PROCEEDINGS OF THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

VOLUME 3 WASHINGTON, D. C., JULY, 1936 NUMBER 2

Observations on the chemical nature of the cuticle of *Ascaris lumbricoides* var. *suis*. B. G. CHITWOOD, U. S. Bureau of Animal Industry.

INTRODUCTION

Up to the present time the chemical nature of the cuticle of nematodes has not been settled, since several workers have investigated the subject and each has reached a separate conclusion. The terms chitin, keratin, cornein, and cutin have all been applied to substances composing the cuticle. The term cutin was, however, used in a loose sense and was not intended to refer to the compound cutin which is a variety of cellulose found only in plants.

Morphologically, the cuticle of *Ascaris* consists of the following layers: (1) A cortical layer consisting of (a) an external cortical layer and (b) an internal cortical layer; (2) a fibril layer; (3) a matrix layer; and (4) three fiber layers. The above designations for these layers will be followed throughout this paper.

The writer is indebted to Mr. Jacob M. Schaffer of the Biochemic Division, U. S. Bureau of Animal Industry, for valuable assistance during the course of this work.

REVIEW OF LITERATURE

Odier (1823) first proposed the term chitin for the highly insoluble substance forming the exoskeleton of insects and in 1850 Grube applied this term to the cuticle of ascarids. However, Lassaigne (1843) had already pointed out that the external covering of annelids and nematodes was soluble in KOH and was, therefore, not chitin. Sukatschoff (1899) confirmed Lassaigne's observations stating that the cuticle of *Parascaris equorum* was completely soluble in hot 35 per cent KOH, gave a positive Millon reaction, and that all layers *except the cortical layer* were digested after 2 to 3 days in artificial gastric juice at 40° C.

Reichard (1902) found that the cuticle of either fresh or old alcoholic ascarids (species not stated) was insoluble in hot or cold water and that all except the cortical layer went into solution when heated to 140° C. in water in a closed tube. The solute was clear and would not jell if evaporated; with Millon's reagent it gave a finely flocculent white precipitate, part of which turned red on warming; the xanthoproteic and biuret reactions were positive. The non-dissolved portion (cortical layer) also gave a positive reaction to the Millon and xanthoproteic tests, but was negative to the biuret test. The non-dissolved portion was soluble in 1 per cent KOH after superheating in water. The cuticle, excluding the cortical layer, became dissolved in dilute solutions of alkalis and mineral acids after a few hours at 40° C.; the "internal layers" were readily soluble in hot concentrated KOH, while the cortical layer dissolved somewhat more slowly in this solvent. In concentrated HCl the internal layers dissolved in a few hours at room temperature, giving a blue violet color; the cortical layer dissolved after a few days. The internal layers were dissolved by gastric as well as pancreatic juice at 40° C., and the cortical layer which was not digested still gave positive Millon and xanthoproteic tests. The whole cuticle dissolved in KOH in a platinum spoon gave a strong "Hepar reaction."

Flury (1912) obtained for chemical tests the "cuticle" of *Ascaris* by digestion with pepsin under toluol. Presumably the results thus obtained can be referred to as tests of the cortical and fibril layers, since one would expect the other layers to dissolve. Material prepared as above was found to dissolve partially in warm dilute H_2SO_4 and hot KOH, forming a fine fibrous flocculum in either solvent. In concentrated H_2SO_4 the "cuticle" showed very little yellow coloration and after 48 hours a fine fibrous material remained undissolved; the H_2SO_4 solvent remained colorless. The "cuticle" obtained by digestion, as already noted, was heated in 10 per cent HCl and the filtrate neutralized with "soda." The following results were then obtained with the filtrate: Copper salt, no precipitate; $HgCl_2$ in acid, precipitate (Niederschlag); lead acetate, strong precipitate; baryta water, very little precipitate (Niederschlag); copper-mannit, no reduction; xanthoproteic, positive; biuret, positive; Millon, yellow color; Adamkiewicz (cane sugar + HCl), red color (tryptophane); conc. HCl, rose color; Ehrlich—Neubauer (p-Dimethyl-amino-benzaldehyde + H_2SO_4), violet color (carbohydrate).

Further purification for 2 to 4 weeks, using 0.85 per cent salt solution, 0.1 per cent ammonium hydroxide, 0.1 per cent HCl, distilled water, alcohol, and ether, and drying in vacuo resulted in a substance absolutely free from carbohydrates; this solution gave only a weak test for tryptophane. Analysis of this "purified cuticle" gave the following results: Total carbon, 50.01 to 51.13 per cent; hydrogen, 5.56 to 6.62 per cent; nitrogen, 15.73 to 16.73 per cent; oxygen, 21.10 to 23.80 per cent; and sulphur, 4.12 to 4.607 per cent. On the basis of these data Flury concluded that the "cuticle" was composed of keratin.

Magath (1919) made a study of the cuticle of *Camallanus americanus*, the material being obtained by scraping the cuticle free from the underlying tissue, washing in distilled water and drying to a constant weight in an oven at 70° C. This entire cuticle was found to be insoluble in dilute mineral acids but soluble on standing in concentrated H_2SO_4 or HNO_3. Hot concentrated acids and cold caustic alkalis (even 1 per cent) dissolved the cuticle upon standing, and more readily when heated to 70° C.; it was soluble in NH_4OH. Tests for uric acid, creatine and urea were negative. No reduction was obtained with Fehling's solution, either before or after hydrolysis. Xanthoproteic, Hopkins-Cole, and unoxidized sulphur tests were positive; the biuret test gave a deep purple color; Millon's reagent gave either a weak red color or a negative reaction; after hydrolysis a test for tyrosine was negative. The cuticle was boiled several hours in water, the filtrate precipitated in alcohol, filtered and dried. Tests on this material showed no free acid but gave a positive Hopkins-Cole test for tryptophane; cystine was found in the filtrate. Using the entire cuticle for determinations, the total nitrogen was found to be 16.90 to 17.04 per cent, and the total sulphur 1.16 to 1.25 per cent. Magath also stated that the cuticle swelled in acetic acid, was partially soluble in boiling water, and that Reichard (1902) and Berge and Berge (1915) found it to be digested by enzymes. As previously noted, Reichard found that only the internal layers of the cuticle were digested. Berge and Berge made no specific statements with reference to the cuticle being digested by enzymes. Magath called the substance composing the cuticle cornein but this was due to a misinterpretation of the headings in Reichard's paper; Reichard applied this term only to the horny substance of corals.

Mueller (1929) obtained cuticle of *Ascaris lumbricoides* by scraping the musculature from the body wall and washing the remaining cuticle in distilled water. He then autoclaved it for 20 hours at 12 pounds pressure. The inner layers were dissolved, leaving the outer layer which was then removed and dried at minus 5 pounds pressure at a temperature of 100° C. The solution when evaporated at the same temperature and pressure as above yielded a glue which gave none of the reactions of proteins. Mueller also found that with whole cuticle Millon's test for tyrosine was positive; no characteristic swelling in acetic acid was observed. Total nitrogen and sulphur determinations on the

internal layers gave the following results: Nitrogen, 16.08 to 16.10 per cent; sulphur, 0.818 to 0.823 per cent; total nitrogen determination of the cortical layer showed 15.86 to 15.97 per cent. On the basis of these observations he concluded that the cuticle was composed of two substances neither of which could be identified with any known protein group.

SOURCE OF MATERIAL USED IN WRITER'S INVESTIGATIONS

The ascarids from which the material used in the experiments reported in the present paper were collected from pigs at Baltimore, Md. The cuticle was obtained by snipping off the two extremities of the worms, slitting them lengthwise, and passing them through a pair of forceps to remove the viscera. The specimens were then placed on a glass plate and the hypodermis and musculature were removed by scraping. The scraping was best accomplishd by having some water on the plate, stretching the body wall of the worm out flat and then pushing a glass slide across the body wall lengthwise. When all musculature and hypodermis were removed the cuticle was transparent. The cuticle of about 200 ascarids was collected in this manner. This material was washed, drained, and placed in an electric refrigerator for 12 hours at about 10° C. to allow the superficial moisture to evaporate. The material thus obtained weighed 26 gm; it was dried in a vacuum desiccator over H_2SO_4 at 0° C. for the first 24 hours, at 10° C. for 76 hours, and at room temperature for 24 hours, these operations resulting in the reduction of the mass to a constant weight of 5.67 gm.

PRELIMINARY OBSERVATIONS

1. *Enzyme tests.*—Small pieces of cuticle were placed in artificial gastric juice (Pepsin, 4 gm, conc. HCl, 10 cc, distilled water 900 cc) under toluol; at the end of 48 hours at 40° C. the cortical and fibril layers remained undigested; digestion experiments carried out in a similar manner with artificial pancreatic juice (pancreatic extract No. 1, see below) caused no visible change in the cuticle. Because of the disagreement of the writer's results with the findings of previous authors, samples were submitted to Dr. Walter S. Hale of the U. S. Bureau of Chemistry and Soils who kindly repeated the digestion tests with various extracts; Dr. Hale also carried out all later experiments involving digestion with pancreatic enzymes, and the writer is grateful to Dr. Hale for this help. In these tests, four different extracts were used: (1) Strong trypsin, prepared by Northrup's process, (According to Dr. Hale, 1 cc of this enzyme sample gives 1.3 cc split of $N/_{10}$ alcoholic KOH on a buffered substratum of casein); (2) strong trypsin plus enterokinase; (3) Fairchild's trypsin; and (4) a 75 per cent glycerin extract of dried hog pancreas. With all of these enzyme extracts NH_4OH and NH_4Cl both in molar concentration were mixed and used as a buffer; the experiments were run at pH 8.4. These extracts will hereafter be designated as pancreatic extracts Nos. 1, 2, 3, and 4.

The results of these tests are briefly summarized as follows: Pieces of cuticle were kept for 4 days in pancreatic extracts Nos. 1 and 4 with no visible effect; in pancreatic extract No. 2 the matrix layer was removed by the process of digestion, thus separating the cuticle into 2 parts; in pancreatic extract No. 3 the matrix, cortical and fibril layers were completely digested but the fiber layers appeared unchanged. These enzyme tests indicate that the cuticle of *Ascaris* is composed of at least three distinct substances, namely, one in the cortical-fibril layers, another in the matrix layer, and a third in the fiber layers.

2. *Solubility tests.*—The cuticle was found to be insoluble in alcohol, ether, chloroform, sat. $(NH_4)_2SO_4$, and glacial acetic acid. The other solubility tests shown in table 1, indicate that in general the fiber and matrix layers are the most soluble in solutions of CH_3COOH, HCl, H_2SO_4, HNO_3, NaOH, NH_4OH, and KOH, and the fibril and cortical layers least soluble in the above solvents. Boiling the cuticle in water, if carried out over an extended period (10 to 12

hours), causes partial solution of the matrix and fiber layers. At least 2 distinct substances can be separated on the basis of solubility. The designation ± as applied to the cortical-fibril part of the cuticle indicates that the fibril layer is at least partially dissolved, but it is difficult to ascertain whether any part of the cortical layer is dissolved.

TABLE 1.—*Solubility tests on whole dried cuticle*[1]

Reagent	Solubility (in hours) of cortical and fibril layers at—		Solubility (in hours) of matrix and fiber layers at—	
	Room temp.	80°C	Room temp.	80°C
10% CH_3COOH	—	—	—	± 1
Conc. HCl	+24	+½	+ 1	+½
Conc. H_2SO_4	—	± — 1	+24	+ 1
10% H_2SO_4	—	± — 1	+24	+ 1
Conc. HNO_3	±24	+½	+½	+¼
10% conc. HNO_3	—	± +½	—	±½
50% NaOH	—	± 1	—	+ 1
20% NaOH	—	+½	—	+½
10% NaOH	±24	+½	+ 1	+½
5% NaOH	± 6	+½	+ 1	+½
Conc. NH_4OH	—	—	—	+ 1
10% conc. NH_4OH	—	—	—	+ 1
50% KOH	—	±½	—	+½
10% KOH	± —24	±½	+½	+½
5% KOH	±24	+½	+½	+½
1% KOH	± — —24	±½	+ 1	+½

[1]The sign + indicates complete solubility; ± indicates partial solubility; ± — indicates slight solubility; ± — — indicates very slight solubility.

3. *Color reactions.*—The color reactions were carried out according to the directions given by Cole (1933). Tests dealing with minute quantities of the various segregates were made by using fractions of the amounts specified. All color tests were checked against known positive and known negative controls. For each test the cuticle was dissolved in an appropriate reagent, i.e., 5 per cent NaOH, 10 per cent HCl, 10 per cent H_2SO_4, according to the reagents required in the tests. Tests involving whole cuticle gave all positive reactions as follows: Xanthoproteic, mercuric nitrate (modified Millon), aldehyde, biuret, arginine (Sakeguchi), lead acetate in 5 per cent NaOH (for unoxidized sulphur), Molisch, and barium chloride (for oxidized sulphur).

4. *Qualitative tests.*—Preliminary qualitative tests were made in order to obtain information as to the proteins which might be present in the cuticle. These tests were made on materials prepared in the same manner as those described elsewhere in this paper under the heading of "analysis." Such tests indicated the presence of a contaminant in the form of a water soluble carbohydrate. This carbohydrate probably was glycogen, since v. Kemnitz (1912) has shown that in *Ascaris* the hypodermis is the chief source of glycogen. These tests also indicated the possible presence of an albumin, a glucoprotein, a fibroid, a collagen, and a keratoid.

ANALYSIS

Following the preliminary survey of the proteins which might possibly be present in the cuticle, an attempt was made to isolate the various protein fractions on the basis of their solubility in water, in one-half saturated lime water, and by pancreatic and peptic digestion. The albumins and water soluble carbohydrates were obtained in the water fraction (hereafter referred to as segregate 1). In order to obtain other segregates these substances were removed by

prolonged washing in running water. The glucoproteins (segregate 2) were obtained in the lime-water fraction by the method described by Gies and his coworkers (1901-1902); glucoproteins were removed from other segregates in the same manner. The fibroid material (segregate 3) was isolated by digestion in pancreatic extract No. 2 and removed from subsequent segregates by the same process. The collagenous material (segregate 4) was obtained by heating the residue in water after removal of segregates 1, 2, and 3, or by obtaining the residue after digestion in pancreatic extract No. 3. The keratoid material (segregate 5) was obtained as a solid either after removal of segregate 4 by boiling, or by peptic digestion.

The large apparent loss of materials in the analyses caused the writer to weigh, redry and reweigh the stock dried cuticle which had been stored in a bottle. Although the material appeared completely dry, it lost 10 per cent in weight on further drying, hence it is very distinctly hygroscopic; because of this fact it was necessary to make correction for this difference in each experiment.

Segregate 1

Procedure.—The weighed dry cuticle was placed in a flask of distilled water containing toluol; at intervals of 24 hours the fluid was drained off, filtered into a weighed beaker, dried over a steam bath in an air blast, and in a desiccator; the beaker was then weighed and the weight of the residue computed.

Results.—One sample of 200 mg (corrected weight) of cuticle yielded 50 mg of residue or 25 per cent.

Reactions.—A 0.72 per cent solution made by dissolving 36 mg of residue in 5 cc of distilled water gave the following color tests: Xanthoproteic, intense positive; aldehyde (for tryptophane), intense positive; mercuric nitrite (for tyrosine), intense positive; biuret, moderately strong positive, violet; arginine, trace; basic lead acetate (for cystine), strong positive; Molisch (for carbohydrate), positive; barium chloride (for sulphate), negative. Precipitation tests were as follows: ½ sat. $(NH_4)_2SO_4$, negative; sat. $(NH_4)_2SO_4$, positive; NH_4OH, negative; alcohol, positive; tannic acid, positive; dilute acetic acid, negative; picric-alcohol, positive. Reaction slightly alkaline to litmus.

Five cc of a 0.2 per cent solution of segregate 1 acidified with 0.2 cc of 1 per cent glacial acetic acid coagulated at 50-60°C.; it was then filtered and the filtrate coagulated at 75-85°C.; the filtrate from the second coagulation on boiling yielded no further coagulation. This filtrate gave negative color reactions except slight tests for arginine, carbohydrate, and dipeptide linkage. Picric-alcohol gave no precipitate; sat. picric acid gave a precipitate, soluble on heating.

Discussion.—Segregate 1 contained in the filtrate, after coagulation, some free carbohydrate mixed with a substance giving the tests for gelatin (compare with segregate 4). The coagulable portion, which appears to constitute the bulk of segregate 1, gave all reactions of albumins listed by Cole (1933). The different coagulation points indicated that possibly two different albumins may be present.

Segregate 2

Procedure.—The weighed dry cuticle was washed in running water for 3 days, extracted for 3 days with several changes of one-half sat. lime water, centrifuged, and the supernatant fluid drawn off and filtered. The filtrate was neutralized with 10 per cent conc. HCl (0.2 per cent HCl as recommended by Gies and coworkers diluted the solution to too great an extent); this produced a very slight turbidity. The material was then centrifuged, the supernatant fluid drawn off and the residue washed in 0.2 per cent HCl, then in water and finally in alcohol; practically all the residue was redissolved by this process. The washings were concentrated by evaporation. The supernatant fluid from the first centrifuging was saved.

Results.—No method of separating this segregate from $CaCl_2$ and $Ca(OH)_2$ has been developed. No estimation of the quantity of this segregate was made.

Reactions.—The filtrate, supernatant fluid and washings all gave identical tests: All color tests were positive; the material was coagulated by tannic acid but not by boiling in acidified solution; it was precipitated by picric-alcohol, and $Hg(NO_3)_2$, but not by acetic acid and only partially precipitated by 0.2 per cent HCl and 95 per cent alcohol.

Discussion.—The results are, admittedly, not satisfactory. They seem to indicate the presence of a glucoprotein (mucoid); the quantity of precipitate with tannic acid seeming to indicate that this substance is present in considerable quantity; however, if this be so, it should have been precipitated when the solution was neutralized with HCl. The only explanation that may be offered is that the solution was too dilute. This is the explanation offered by Hawk and Gies (1901) for the negative results obtained by Young (1892) who extracted bone in an excess of lime water (100 cc per gram of substance) and upon neutralization obtained no mucoid. Hawk and Gies obtained satisfactory results when they used but 2 to 5 cc of lime water per gram of material. Unfortunately, this small quantity of fluid could not be used in the present experiment as it would not cover 0.5 gm of cuticle, and for that reason the writer used 30 to 100 cc of lime water for samples of from 0.5 to 2 gm.

Segregate 3

Preparation.—The weighed dry cuticle was washed for 3 days in running water and the insoluble portion washed for 3 to 5 days in lime water; the remaining insoluble portion was washed for 12 hours in several changes of 0.2 per cent HCl, and then washed for 12 hours in several changes of distilled water, and finally digested for 5 days in pancreatic extract No. 2 under toluol. This material was filtered into a weighed beaker and the filtrate evaporated to dryness over a steam bath in an air blast followed by desiccation in vacuo, and the beaker weighed. As a control an equal quantity of pancreatic extract No. 2 and toluol was filtered and dried as above. The residue of segregate 3 was computed by subtracting the weight of residue obtained in the control sample from weight of residue obtained in the experimental sample.

Results.—Following the procedure given above, (1) 500 mg (450 mg corrected weight) of cuticle yielded 159 mg of segregate 3, or 35 per cent; (2) 500 mg (450 mg corrected weight) of cuticle yielded 140 mg of segregate 3 or 31.1 per cent (amount of enzyme used in (1) and (2) 10 cc active trypsin, 10 cc enterokinase, 5 cc buffer); (3) 2,000 mg (1,800 mg corrected weight) of cuticle yielded 407 mg of segregate 3, or 22.5 per cent (amount of enzyme used, 30 cc active trypsin, 30 cc enterokinase, 15 cc buffer).

Reactions.—Residue (containing pancreatic extract) and pancreatic extract control were each dissolved in distilled water to make 1 per cent solutions. The following color tests were the same in both the experiment and control solutions: Xanthoproteic, intense positive; aldehyde, intense positive; nitrite, intense positive; Molisch (hydrolized in 10 per cent HCl), positive; barium chloride, positive; arginine, negative; biuret, negative. The sulphide test on the experiment solution was positive, on the control solution negative. Dilution of both experiment and control solutions to 0.25 per cent gave a sharp differentiation, the experimental solution retaining strong xanthoproteic, aldehyde, nitrite and sulphide reactions and the control solution giving a very weak aldehyde reaction and only slight nitrite and xanthoproteic reactions; the sulphate and Molisch reactions were identical for the experiment and control solutions.

Discussion.—It appears that the carbohydrate, as indicated by the Molisch test, was introduced with the enzyme. This view is supported by the fact that a mixture of segregates 3 and 4 obtained by peptic digestion gave a negative Molisch test. It also appears that this segregate contains both tryptophane and tyrosine, and while it is possible that some of these substances were introduced with the enzyme, they were introduced in a larger measure by the cuticle.

These observations are supported by tests on a mixture of segregates 3 and 4 obtained by peptic digestion and on the basis of these observations, together with the solubilities of the matrix layer from which this segregate is derived, the writer concludes that the substance comprising the matrix layer should be classified with the fibroids, although none of the other fibroids give a positive sulphide reaction. Keratoid affinity is excluded because of the very soluble nature of the material and its digestion in artificial gastric juice; the only similarity to a keratoid is in the strong sulphide reaction. Collagen affinities are excluded because of its digestion in pancreatic extracts, its strong xanthoproteic, aldehyde, and nitrite reactions, and the fact that it does not form a gelatin.

It differs from elastin and reticulin in that these substances give a weak nitrite test (for tyrosine) and a negative aldehyde test (for tryptophane); it differs from fibroin only in giving a positive sulphide reaction. The term matricin is proposed by the writer for the substance forming the matrix layer, from which segregate 3 is derived, because it appears to be clearly separable from the substances mentioned above.

Segregate 4

Preparation.—The weighed dry cuticle was washed for 3 days in running water; the insoluble portion washed for 3-5 days in lime water; the remaining insoluble portion washed for 12 hours in several changes of 0.2 per cent HCl and then washed for 12 hours in several changes of distilled water; it was then digested for 5 days in pancreatic extract No. 2 under toluol. The material was filtered and the solid washed for 12 hours in several changes of distilled water. Two procedures were then followed:

Procedure A. Material obtained by treating cuticle as outlined under above was heated for 5 to 10 hours in distilled water over a steam bath, centrifuged, the fluid drawn off and filtered into a weighed beaker; the filtrate was dried over a steam bath with an air blast, then in a desiccator, and the residue and beaker weighed. Distilled water was added to the insoluble portion and the procedure, as given above, repeated 5 times, and then 10 per cent acetic acid was added before heating. The total segregate 4 was computed by totaling the residue contained in the several fractions.

Procedure B. Material obtained by treating cuticle as outlined under the heading "preparation" was digested in pancreatic extract No. 3, centrifuged, and the solid washed for 12 hours in several changes of distilled water; the solid was hydrolized by heating for 12 hours in distilled water over a steam bath; it was dried in a weighed beaker over a steam bath in an air blast, then in desiccator, and weighed.

Results.—Following procedure A, 500 mg (450 mg corrected weight) of cuticle yielded 122 mg of residue, or 27.1 per cent (amount of pancreatic extract No. 2 used, 10 cc active trypsin, 10 cc enterokinase, 5 cc buffer); 2 gm (1.8 gm corrected weight) of cuticle yielded 522 mg of residue, or 29 per cent (amount of pancreatic extract No. 2 used, 30 cc enterokinase, 30 cc trypsin, 15 cc buffer). Following procedure B, 500 mg (450 mg corrected weight) of cuticle yielded 54 mg of residue, or 12 per cent (amount of pancreatic extract No. 3 used, 60 mg Fairchild's trypsin, 3 cc buffer, 30 cc water). Residue clear, transparent, amber. Residue from water fractions, when dissolved in water to make a 4 per cent solution, forms a soft jell at 16°C., a hard jell at 0°C.

Reactions.—The residue obtained by each of the above procedures was dissolved in distilled water to make a 0.4 per cent solution. Tests on these solutions were as follows: Xanthoproteic, negative; aldehyde, negative; mercuric nitrite, negative; Molisch, negative; arginine, positive; biuret, positive; sulphate, negative; sulphide, positive; Berrár test for gelatin (at 0.10 per cent), positive; 95 per cent alcohol, precipitate; tannic acid, precipitate; 0.2 per cent HCl, no precipitate; 10 per cent glacial acetic acid, no precipitate; glacial acetic acid, no precipitate.

Discussion.—The distilled water fractions of segregate 4 obtained by procedure A include only the fiber layers. The acetic acid fraction contains the internal cortical and fibril layers, as well as remnants of the fiber layers which failed to dissolve in distilled water. An extremely fine hair-like mass representing the cortex *sensu restricto* (see segregate 5) remained after treatment with hot 10 per cent acetic acid. The ease with which the various cuticular layers are dissolved by hot water or hot acetic acid is entirely dependent upon prior treatment. Under "Solubility tests" in our preliminary observations, it was noted that the cortex and fibril layers were not affected by hot 10 per cent acetic acid. Treatment in lime water and pancreatic extract appears to have changed their solubility. The acetic acid fraction of segregate 4 differed in no color reaction from the other fractions. The Berrár test was positive for gelatin except that the picric acid precipitate did not completely dissolve on heating; the Stokes, tannic acid, and alcohol tests were positive. Segregate 4 (by procedure B) contained only the fiber layers. The internal cortical and fibril layers were removed by pancreatic extract No. 3, and part of the fiber layers might conceivably have been included in that fraction. The segregate removed in pancreatic extract No. 3 in an experiment using 500 mg (450 mg corrected weight) yielded 76 mg of residue or 16.9 per cent of the total weight of the cuticle. It has not been determined whether the fibril and internal cortical layers are identical in composition with the fiber layers, but the difference in solubility and digestibility indicate that they are not. Thus far the substances comprising the internal cortical and fibril layers have not been obtained pure, since, by procedure B, they are mixed with pancreatic enzyme, the products of the external cortical layer and possibly parts of the fiber layers. By procedure A the internal cortical and fibril layers are mixed (in the acetic acid fraction) with remnants of the fiber layers.

On the basis of these tests, at least, the fractions of segregate 4 containing only the fiber layers must be considered a gelatin. Since no precipitate was obtained on the addition of picric-alcohol, this indicates, according to Berrár (1912), the absence of other proteins; lack of complete solubility in heated picric acid occurred only in the acetic acid fraction, and probably indicates partial hydrolysis of the gelatin. Of the proteins which yield a jell, sericin differs from segregate 4 in containing a large amount of tyrosine, and glucoproteins differ in containing a carbohydrate group. One keratoid (that occurring in whalebone) yields a jell only when boiled with acetic acid but like other keratoids it differs from segregate 4 in giving intense mercuric nitrite, xanthoproteic and aldehyde reactions. Though segregate 4 differs from gelatin in the presence of sulphides, it must be placed in that group together with sericin. The term ascarogelatin is proposed for the above described gelatin and ascarocollagen for the material from which it was derived on hydrolysis.

Segregate 5

Procedure A.—The solid material obtained following procedure A given under segregate 4 was washed with distilled water, centrifuged, the fluid drawn off, and the residue dried in a weighed beaker over a steam bath and in a desiccator.

Procedure B.—Carbohydrates, albumins and glucoproteins were removed as described under segregate 2, the first with running water and the second with ½ sat. lime water. The solid was washed in 0.2 per cent HCl toluol and digested for 6 days at 40° C.; 50 cc additional gastric juice was added on the third, fourth, and fifth days; the fluid was then removed by centrifuging; and the solid thus obtained was washed in distilled water, placed in a weighed beaker and dried over a steam bath and in a desiccator.

Results.—Following procedure A, 500 mg cuticle (450 mg corrected weight) yielded 11 mg of residue or 2.4 per cent of the total weight of cuticle and 2.0 gm cuticle (1.8 gm corrected weight) yielded 37 mg of residue or 2.0 per cent of total weight of cuticle; following procedure B, 1.0 gm cuticle (900 mg corrected weight) yielded 20 mg of residue or 2.2 per cent of total weight of cuticle.

Tests.—The solid material prepared as above, was dissolved in conc. HNO_3, 10 per cent KOH, and 10 per cent H_2SO_4 for color tests, with results as follows: Xanthoproteic, positive; mercuric nitrite, positive; aldehyde, positive; arginine, moderately weak positive; biuret, red; Molisch, negative; sulphide, very intense positive.

Discussion.—Segregate 5 as prepared by both procedures is the pure external cortical layer; it is a solid composed of fine hair-like strands. The extreme insolubility of these strands corresponds entirely to the preliminary solubility tests (see Table 1). It agrees with keratin in its insolubility, in its color tests and its resistance to peptic digestion. There is only one apparent difference between segregate 5 and keratin: Segregate 5 is digested by pancreatic juice (pancreatic extract No. 3) while, generally, keratin is said not to be acted upon by pancreatic enzymes. The indigestibility of keratin has, however, been questioned by Dreaper (1914) who states that this is probably erroneous since keratin is used in coating pills intended to act on the small intestine.

Summary of Analyses

Five quantitative experiments were carried out with the following results:

Experiment I yielded 25 per cent of segregate 1.

Experiment II yielded 35.1 per cent of segregate 3; 29.2 per cent of segregate 4, obtained by procedure A (19.5 per cent in water fractions and 9.7 per cent in acetic acid fractions); and 2.4 per cent of segregate 5. Total yield 66.7 per cent.

Experiment III yielded 31.1 per cent of segregate 3; 28.9 per cent of segregates 4 plus 5, obtained by procedure B (12 per cent in fraction not digested by pancreatic extract No. 3 and 16.9 per cent in fraction digested by pancreatic extract No. 2). Total yield 60.0 per cent.

Experiment IV yielded 22.6 per cent of segregate 3; 29.0 per cent of segregate 4, obtained by procedure A (19.8 per cent in water fractions and 9.2 per cent in acetic acid fraction); and 2.0 per cent of segregate 5. Total yield 53.6 per cent.

Experiment V yielded 65.2 per cent of segregates 3 plus 4 (by peptic digestion); 2.2 per cent of segregate 5. Total yield 67.4 per cent.

As indicated by experiment I, experiments II, III, IV, and V should account for 75 per cent of the weight of the cuticle minus the weight of segregate 2. The fact that experiments II to V accounted for 53.6 to 67.4 per cent of the total weight of cuticle is admittedly too wide a variation. Segregates 4 and 5 together accounted for 28.9 to 31.6 per cent of the weight and this appears reasonably accurate. The higher figure for segregate 3, 35 per cent obtained in experiment II, is probably correct since it is confirmed by subtracting the figure for segregate 4, approximately 29 per cent obtained in experiments II and IV, from the figure for segregates 3 plus 4, 65 per cent, as obtained in experiment V. The variation in actual yield of segregate 3 is due to incomplete digestion with consequent loss in filtering which always occurred in the preparation of segregate 3.

SUMMARY

The cuticle was found to be composed of five substances which were segregated by physical and chemical means. Segregate 1 contained albumins and segregate 2 contained a substance which was probably a glucoprotein (mucoid), neither of which correspond to known morphological entities. Segregate 3 contained a fibroid for which the term matricin is proposed; it corresponds to the matrix layer. Segregate 4 contained a member of the collagen group for which the term ascarogelatin is proposed, the non-hydrolized form from which it was derived being termed ascarocollagen; it corresponds to the fiber, interno-cortical and fibril layers. Segregate 5 is a keratin and corresponds to the external cortical layer.

TABLE 2.—*Comparative table showing the characteristics of albuminoids*

	Cold Water	Hot Water	Peptic Digestion	Panc. Digestion	Xanthoproteic	Tyrosine	Tryptophane	Sulphide
COLLAGENS								
Gelatin	+	+	+	−	+ (slight)	+(slight) or −	−	−
Collagen	−	+	+	−	+ (slight)	+(slight) or −	−	−
Serecin	−	+	+	−	+	+	+	−
Ascarogelatin	+	+	+	−	+ (slight)	−	−	+
Ascarocollagen	−	+	+	−	+ (slight)	−	−	+
FIBROIDS								
Elastin	−	−	+	+	+	±	−	−
Reticulin	−	−	+	+	+	±	−	−
Fibroin	−	−	+	+	+	+	+	−
Matricin	−	−	+	No. 2 and No. 3 +	+	+	+	+
KERATOIDS								
Keratin	−	−	−	±	+	+	+	+
Ascaris keratin	−	−	−	No. 3 +	+	+	+	+

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A new species of cestode, *Davainea meleagridis* (Davaineidae) from the turkey, with a key to species of *Davainea* from galliform birds. MYRNA F. JONES, U. S. Bureau of Animal Industry (Transferred to National Institute of Health).

Small cestodes collected from a turkey, *Meleagris gallopavo*, in November 1930, represent a new species of *Davainea*, s. st. The turkey was obtained from a market in Washington, D. C., and presumably came from nearby Maryland or Virginia.

Davainea meleagridis, n. sp. (fig. 15, A-E)

Description.—Mature specimens up to 5 mm long by 950μ wide and composed of 17 to 22 segments. Scolex 147 to 175μ wide; suckers 42 to 50μ in diameter with 4 to 6 rows of hooklets, the longest about 5μ long. Rostellum 70 to 85μ wide with a double row of about 100 to 130 hooks, 8 to 10.5μ long. Musculature of strobila weak. Excretory vessels never prominent, most transverse sections studied failing to show 4 definite longitudinal vessels. Genital pores usually regularly alternate, but in a few specimens irregularly alternate. Genital atrium 35 to 40μ deep in moderately contracted segments. Testes in posterior half of segment, 20 to 26 in number, up to 70μ in diameter. Cirrus pouch large, extending at least to midline of mature segments, typically about 245μ long by 98μ wide in mature segments, varying in one strobila from 175μ long by 98μ wide to 273μ long by 87μ wide. Cirrus with delicate spines, prominent in cirrus pouch when withdrawn; extended cirrus about 450 to 500μ long, with cirrus pouch considerably reduced in size. Vagina posterior to cirrus pouch, with heavy wall lined with fine cilia; vagina wide near genital atrium but lacking a saccular appendage such as that in *Davainea tetraoensis*. Seminal receptacle median in segment, spherical, about 42 to 47μ in diameter. Ovary bilobed, diffuse. Vitelline gland posterior to ovary, slightly lobed, transversely elongate. Uterus visible in twelfth segment. Eggs single, in capsules, about 28 to 35μ in diameter; oncospheres about 25 to 28μ in diameter; embryonal hooks 14 to 18μ long.

Host.—*Meleagris gallopavo.*

Location.—Duodenum.

Distribution.—Vicinity of Washington, D. C.

Type specimens.—U. S. N. M. Helm. Coll. No. 30606.

Of the 8 species of *Davainea*, s. st., previously described, 2 species occur in charadriform birds and 6 species in galliform birds. *D. meleagridis* differs from both of the species occurring in charadriform birds in having a greater number of segments and also a greater number of testes, 10 to 12 testes occurring in *D. minuta* Cohn, 1901, and only 4 testes occurring in *D. himantopodis* Johnston, 1911. The following provisional key may be used to distinguish *D. meleagridis* from species of *Davainea*, s. st., from *galliform* birds; a satisfactory key to this group is difficult to make because several of the species have been incompletely described.

Key to species of Davainea, s. st., described from galliform birds

1. Strobila with less than 15 segments 2
   Strobila with more than 15 segments 4
2. Head large, 600 to 700μ wide; testes about 40 in number. *D. paucisegmentata* Fuhrmann, 1909
   Head 130 to 200μ wide; testes 12 to about 30 3
3. Vagina with accessory sac; genital pore not at anterior angle of segment margin ..... *D. tetraoensis* Fuhrmann, 1919
   Vagina without accessory sac; genital pore at anterior angle of segment margin ..... *D. proglottina* (Davaine, 1860)
4. Genital pores unilateral (not according to generic diagnosis) *D. tragopani* Southwell, 1922, (syn. ? *D. facile*)
   Genital pores alternate 5
5. Testes about 50; genital pores regularly alternate ..... *D. nana* Fuhrmann, 1912
   Testes less than 50; genital pores regularly or irregularly alternate 6
6. Cirrus pouch not extending to midline of segment; vagina not wide and without heavy wall ..... *D. andrei* Fuhrmann, 1933
   Cirrus pouch extending at least to midline of moderately contracted segments; vagina wide, with heavy wall ..... *D. meleagridis*

In addition to the characters given in the key, *D. meleagridis* is further distinguished from *D. tragopani* by its greater number of testes: 20 to 26 as compared with 6 to 7 (? 9) testes. *D. meleagridis* is further distinguished from *D. nana* by its smaller rostellar hooks; hooks 8 to 10.5μ long as compared to hooks 18μ long. *D. meleagridis* is somewhat similar to *D. andrei*, described from *Perdix perdix* by Fuhrmann in 1933; however, in addition to the proportionately larger cirrus pouch of *D. meleagridis*, it is actually larger in segments of a similar state of maturity, the cirrus pouch of *D. meleagridis* varying in size from 175 to 273μ long by 98 to 105μ wide and that of *D. andrei* varying from 160 to 180μ long by 60μ wide. Further, the genital pores of *D. meleagridis* are usually regularly alternate, only a few specimens exhibiting irregularly alternate pores, while the genital pores of *D. andrei* have been described as being definitely irregularly alternate; the variation in position of genital pores in *D. meleagridis* would suggest, however, that in this group this character is of limited specific value. The embryonal hooks of *D. meleagridis* are smaller, being 14 to 18μ long, while those of *D. andrei* are 22 to 23μ long. Unfortunately, the rostellar hooks of *D. andrei*, have not been described so that a comparison of hooks for these two species is not possible. As indicated in the key, the greater size and heavier ciliated wall of the vagina of *D. meleagridis*, as compared with that of *D. andrei* are important in distinguishing the two species.

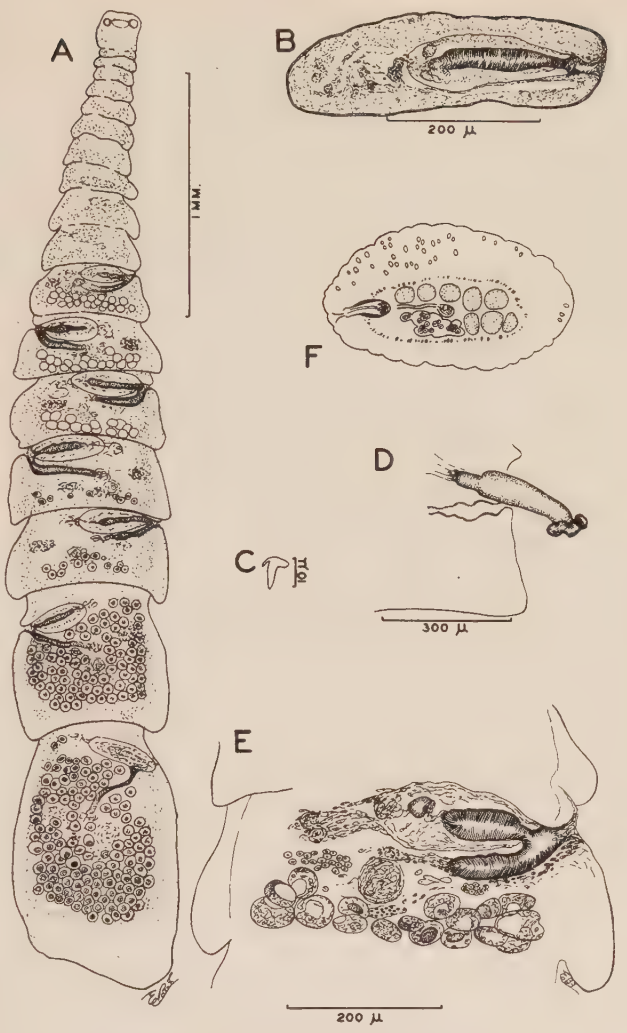

FIG. 15.

A-E—*Davainea meleagridis*. A—Whole specimen, toto mount. B—Transverse section of mature segment. C—Rostellar hook. D—Everted cirrus. E—Frontal section of mature segment. F—*Davainea andrei*, transverse section of mature segment (after Fuhrmann, 1933).

It is probable that a study of additional material of various species of *Davainea*, or, in some instances, a further study of type specimens would make it possible to complete certain specific descriptions and thereby permit a more

satisfactory comparison of species now included in the genus. The following list of species and of certain structures of each which have not been described, so far as the writer is aware, is included as of use in the study of appropriate material whenever it is available:

D. andrei: Rostellar and acetabular hooks; number of testes (indefinite).

D. minuta: Number of rostellar hooks; number of segments of complete strobila; completely formed eggs, length of embryonal hooks.

D. nana: Type of vagina; completely formed eggs, length of embryonal hooks.

D. paucisegmentata: Rostellar and acetabular hooks; length of embryonal hooks; position of genital pores described as unilateral by Fuhrmann (1909, Result, Swed. Zool. Exped. Egypt, pt. 3, no. 27, p. 2) but figured as regularly alternate by Joyeux and Baer (1928, Collect. Soc. Path. Exot., Monog. 2. Cestodes, pp. 17-54).

D. tetraoensis: Completely formed eggs, length of embryonal hooks. The writer has observed ripe segments of specimens, identified as *D. tetraoensis*, from the ruffed grouse (*Bonasa umbellus*), and found the following characters: Egg capsules (in mounts) 35 to 42μ in diameter, outer egg membranes 28 to 34μ in diameter; oncospheres oval, 17μ by 25μ or spherical, 21 to 25μ in diameter; embryonal hooks 12 to 14μ long. Descriptions of eggs of specimens from the type host, *Tetrao urogalli*, would be of interest. After reexamining the specimens of *Davainea* from the ruffed grouse, *Bonasa umbellus*, in the U. S. N. M. Helminthological Collection, it is concluded that an earlier record of *D. proglottina* from the ruffed grouse is erroneous; all complete specimens are now regarded as *D. tetraoensis* and the immature or incomplete specimens are determined as *Davainea* sp. or *D. ? tetraoensis*.

SUMMARY

A new species of cestode, *Davainea meleagridis*, is described from the turkey, *Meleagris gallopavo*. There is included a key to the species of *Davainea*, s. st., from galliform birds, a list of structures which are undescribed for certain incompletely described species of *Davainea*, and a description of the eggs of *D. tetraoensis* from the ruffed grouse, *Bonasa umbellus*. An earlier identification by the writer of *D. proglottina* from the ruffed grouse is now considered erroneous, all available specimens of *Davainea* from the ruffed grouse now being regarded as *D. tetraoensis*.

On the species of *Moniezia* (Cestoda: Anaplocephalidae) harboured by the hippopotamus. J. H. Sandground, Department of Tropical Medicine and Museum of Comparative Zoology, Harvard University.

Aside from *Moniezia rugosa* (Diesing, 1850), which was described from 2 South American monkeys, *M. mettami* Baylis, 1934 from the African wart hog, *M.* (*Fuhrmanella*) *transvaalensis* (Baer, 1925) Baylis, 1935 from *Thryonomys* sp. and *M. amphibia* von Linstow, 1901, all other representatives of the genus *Moniezia* have emanated from ruminants. The genus was accredited with a fairly large number of species until recently, but, as a consequence of the critical studies of Gertrude Theiler (1924) and of Taylor (1928), the majority of these species have been sunk in synonomy, it having been shown that the specific characters that had been previously used were, for the most part, subject to such variation that, taxonomically, they lost all significance. Seemingly the only morphological character that exhibits sufficient constancy to warrant its being accepted as a specific criterion concerns the interproglottidal glands upon which, according to Taylor, 2 species are recognizable: *M. expansa* with glands of the saccular or rosette type and *M. benedeni* characterized by the linear arrangement of these glands. A so called "*denticulata*" group of species devoid of interproglottidal glands was recognized by Theiler and, following this worker's lead, by Baer (1927), but the work of both Theiler and Taylor

showed that in *M. alba*, at least, some segments in a strobila may show interproglottidal glands, albeit sometimes very indistinctly. Taylor considers that the absence of these glands is not a good specific character and on this account he synonomyzes *M. alba* with *M. benedeni*. For *M. pallida* Monnig, 1926, from the horse, and the recently described *M. monardi* Fuhrmann, 1933, from an antelope, *Redunca armidarum*, the extension of uterine folds laterally, beyond the excretory tubes, appears to be a good specific character. The broken distribution of the testicular follicles into 2 triangular areas, such as was held to distinguish *M. trigonophora* Stiles and Hassall, 1890, was shown by Theiler to be inconstant, and the several species based on this character are apparently not distinguishable. Yet Baer (1925 and 1927) continued to retain *M. trigonophora* as distinct from *M. expansa*. In von Linstow's description of *M. amphibia*, no mention is made of interproglottidal glands. Concluding that these glands are absent, Theiler included the species in the *"alba"* group of species. Baer (1925) mentions having reexamined v. Linstow's material in the Berlin Museum and finding interproglottidal glands absent, he concurs in placing *M. amphibia* in synonomy with *M. alba*. However, the demonstration of diffuse glands in the type material of *M. alba*, led Taylor to place *M. alba*, together with *M. amphibia* and other similar species in the synonomy of *M. benedeni*. Whether species, such as *M. denticulata*, definitely devoid of interproglottidal glands exist does not appear to have been fully established, but Baer (1927) continues to recognize the species and includes *M. amphibia* under its synonomy. Baylis (1934) reports that in his *M. mettami*, no trace of intersegmental glands could be seen in either toto-mounts or in sections.

Summarizing the preceding arguments, it may be stated that not one of the 3 recent works retains *M. amphibia* as a distinct and separate species, and that each places it in synonomy with a different species, viz: *M. alba* (Theiler), *M. denticulata* (Baer, 1927) and *M. benedeni* (Taylor). In commenting upon *M. amphibia*, Baer (1925, p. 80) questions whether the hippopotamus serves as a host for species of *Moniezia*, and suggests the possibility of a confusion of labels or the writing of *Hippopotamus* for *Hippotragus* (an antelope). All doubts on this question can now be removed for in 1934, the writer, dissecting a young hippopotamus (*H. amphibia*) which had been shot by Dr. Richard P. Strong, leader of the Harvard expedition for the study of Onchocerciasis in the Belgian Congo, on the Lomami River in northern Katanga, found a very intense infection with a species of *Moniezia*. This material, however, appears to differ from von Linstow's in so far as a series of from 18 to 23 rosettes of interproglottidal glands is conspicuously present in practically all segments of several strobilas stained in Ehrlich's haemotoxyln and paracarmine. In the absence of any morphological differences, these tapeworms must be ascribed to *Moniezia expansa* (Rudolphi) Blanchard, 1891. Hence we may conclude that either the specimens described by von Linstow were in such a state of preservation that the interproglottidal glands do not readily take a stain, or that 2 distinct species of *Moniezia* may be found in the hippopotamus.

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Studies on the life history of *Telorchis robustus* (Trematoda: Plagiorchiidae). Wendell H. Krull, U. S. Bureau of Animal Industry.

The life history of *Telorchis robustus*, a common parasite of the land turtle, *Terrapene carolina*, in Maryland, has been determined experimentally and reported briefly in a previous paper (Krull, 1935, Proc. Helminth. Soc. Wash., 2:65). Eggs dissected out of *T. robustus*, obtained from turtles in the vicinity of Beltsville, Md., were used as a basis for the life-history experiments. In another experiment, cercariae from a naturally infected snail were used in infecting laboratory-raised second intermediate snail hosts. The metacercariae from the latter snails were fed to a laboratory-raised turtle, *Terrapene carolina;* the turtle became infected and almost mature specimens of *T. robustus* were recovered, thus establishing the snail as a natural first intermediate host.

Since food seems to be comparatively scarce for these turtles in the spring under conditions prevailing in nature, and since turtles have been observed to be eating snails in semi-flooded flats at that time of year, it is assumed that the turtles become infected during the spring and early summer. Later in the season the turtles are attracted by other food or, because of dryness, they are forced to abandon these low hunting grounds. An account of the life history, together with descriptions and notes on larval stages, follows.

DESCRIPTIONS OF DEVELOPMENTAL STAGES

(1) *Sporocysts.*—Small oval or ovate sac-like structures, usually containing numerous cercariae. Specimens more or less contracted in egg albumen mounting medium without pressure measured 220μ long by 130μ wide to 300μ long by 130μ wide. Younger sporocysts with developing cercariae are able to bend, contract and extend themselves, being more active than the older ones which are distorted by the numbers and activity of the cercariae which they contain. The sporocysts were exceedingly numerous in experimentally infected snails, being distributed along the intestine and embedded in the digestive gland.

(2) *Cercaria.*—A xiphidiocercaria (fig. 16) with simple tail. Measurements of organs are given in table 1. Most or all of body spined; spines large at anterior end of body, diminishing in size posteriorly and becoming minute over posterior part to a level midway between pharynx and acetabulum. Stylet large, 30μ long exclusive of mucilagenous plug; base wider than shoulder area. Suckers and pharynx well developed. Prepharynx as long as pharynx; esophagus somewhat longer; intestinal ceca poorly developed, extending to near posterior end of body. Nine penetration glands, 4 on one side and 5 on the other. Subcuticular glands about 30 in number, opening in a row immediately anterior to acetabulum. Bladder Y-shaped, walls of cornua glandular, and bladder proper muscular; bladder opening through short duct in fold or groove dorsal to base of tail; main pair of excretory tubules joining cornua near bases. Flame cells observed but pattern not clear. Tail joining body in a pocket or vestibule at posterior end of body. Cuticula of the 2 dorsolateral portions of vestibule greatly thickened, each area provided with about 50 long spines projecting into cavity. Primordium of reproductive organs small, at level of acetabulum.

Fig. 16.
Telorchis robustus, cercaria.

TABLE 1.—*Average measurements in microns of organs of cercariae of Telorchis robustus*

Description of specimen	Body		Tail		Sucker Diameter		Stylet		Pharnyx
	Length	Width	Length	Width	Oral	Acetabulum	Length with Plug	Greatest Diameter	Width
Specimens killed and mounted in 10 per cent hot formalin	264	97	186	28	51	47	....	..	15
Living specimens well flattened	375	182	264	43	77	65	35	8	24

The cercaria is quite unlike that of *Cercorchis medius*, a closely related species, described by McMullen (1934, J. Parasitol., 20:248-250, figs. 1-3). The cercaria of *T. robustus* possesses a larger stylet, smaller number of penetration glands and a vestibule containing numerous spines at posterior end; these characters serve to distinguish it from *C. medius*.

OBSERVATIONS ON CERCARIAE AND METACERCARIAE

The cercariae are long lived. When kept in pond water at room temperature some lived for 79 hours, while others began to die after 55 hours.

By allowing the cercariae to swim for an hour or more in a very dilute solution of thionin in distilled water the gland cells and their ducts readily take up the stain. The stained cercariae may be mounted subsequently in water or egg albumin, the latter being the better medium.

While it has been definitely stated in the previous paper (Krull, loc. cit.) that the cercariae penetrate into the soft parts of the snail, subsequent examinations of numerous snails showed that occasionally one snail of a lot exposed to infection acquired a very much heavier infestation than the other snails of the lot. Such highly susceptible snails, when examined, usually showed large numbers and sometimes masses of cysts along the digestive tract, particularly along the esophagus, and in the vicinity of the heart. On the basis of previous observations and conclusions regarding the penetration into soft parts, it is difficult to account for the occasional unusually heavy infestations and the reason why the majority of cysts are localized along the digestive tract. It is suggested that possibly the snails so infected eat many of the cercariae, after which the cercariae penetrate the wall of the digestive tract and encyst. Some support is given to this assumption by certain observations indicating that while most snails first explore their surroundings on being brought into a new environment, an occasional snail begins to eat immediately. The cercariae spend much time in crawling and for this reason they might be ingested by a snail that is feeding.

A cercaria encysts soon after it penetrates the tissue of the host. The cysts are hyaline, comparatively thin walled, about 2μ thick, and are either oval or round. Apparently the cysts do not increase in size to any extent; the average mean diameter of cysts 2 weeks old was 119μ, and of cysts 8 weeks old 125μ.

Although the metacercaria, apparently, does not increase in size, other changes take place. The stylet is extruded within 20 hours after encystment and remains intact in the cyst during the metacercarial life of the parasite. The body spines grow and the spination becomes very distinct, the spines at the anterior end being the largest, as much as 4μ long in metacercariae which have been encysted for a month. The vestibule with the cuticular areas of

large spines at the posterior end of the body is obliterated, becoming a part of the posterior end of the worm. The oral sucker becomes more muscular and increases somewhat in size, while the size of the acetabulum remains about the same. The ceca develop thick glandular walls and become very conspicuous. The openings of the subcuticular glands immediately anterior to the oral sucker become more prominent. Other subcuticular glands with short ducts, not observed in the cercaria, open to the surface of the body. These glands have no regular arrangement; they are quite numerous from the anterior end to the level of the acetabulum, posterior to which they are almost absent. The excretory vesicle becomes distended with refractile granules within 24 hours after encystment; it becomes large and kidney shaped and fills most of the space in the metacercaria posterior to the acetabulum.

ADDITIONAL NEW HOSTS

In addition to the experimental first intermediate host already mentioned in the previous paper, it has been determined experimentally that *Fossaria parva* (Lea) serves as a natural first intermediate host. The infected snail was collected in the vicinity of Beltsville, where heavily infested turtles, *Terrapene carolina*, occur in abundance. Laboratory-raised snails exposed to cercariae from the infested snail became infected; the soft parts containing the cysts were force fed to a small *Terrapene carolina* which had been hatched and raised in the laboratory. Eighty-four specimens of *T. robustus* were recovered from the turtle post mortem. The flukes varied in size, presumably, because the metacercariae were given to the turtle at different times. The possible age of flukes was from 14 to 76 days, but none were mature. The turtle was infected during the winter months, and since the temperatures were far from those assumed to be suitable for development, it is concluded that temperature was responsible for the slow development of the flukes in the turtle and that the growth observed does not necessarily represent natural growth rate during the summer.

Attempts to infect specimens of *Physa halei* as a first intermediate host were unsuccessful. However, this snail was determined to be a suitable second intermediate host, the specimens so infected being snails hatched and raised under laboratory conditions.

SUMMARY

The sporocyst and cercaria of *Telorchis robustus* are described, and the changes which take place when the cercaria has become encysted are reported. *Fossaria parva* (Lea) has been shown to be a natural first intermediate host, and *Physa halei* has been determined as a new experimental second intermediate host.

New terrestrial and aquatic intermediate hosts for *Brachylaemus virginiana* (Dickerson) Krull (Trematoda: Brachylaemidae). WENDELL H. KRULL, U. S. Bureau of Animal Industry.

In a previous paper the writer (Krull, 1934, J. Wash. Acad. Sc. 24: 483-485) showed by experiment that, although *Brachylaemus virginiana*, which ordinarily is rather strictly limited in its definitive host relationship to the opossum, other mammals, such as dogs, cats, and white rats, as well as chickens could be infected. As a consequence of these findings, the writer undertook experiments with a view to elucidating intermediate host relationships of this parasite. In addition to *Polygyra thyroides*, which was known to be an intermediate host of *B. virginiana*, it has been determined by the writer that the European land snail, *Helix pomatia*, and the slug, *Deroceras laeve*, as well as one of the aquatic snails, *Pseudosuccinea columella*, may serve as second intermediate

hosts, since metacercariae completed their growth in these snails. Besides the snails already mentioned, *Helisoma trivolvis* and *Succinea* sp. were infected by the writer, but experiments were discontinued before the metacercariae reached maturity.

The location and behavior of larvae, except for the differences noted and described, were the same as those reported in a previous paper by Krull (1935, Tr. Amer. Micr. Soc., 54: 118-134).

The new snail hosts mentioned below were identified by members of the Division of Mollusks of the U. S. National Museum.

The snails used in the first experiment were specimens of *Helix pomatia*. These were hatched in the laboratory and were derived from a stock of snails from France. The parent snails were examined for cercariae as a precautionary measure and were negative for all trematode parasites. When the laboratory-raised snails were approximately a third grown they were placed for several hours in a covered finger bowl with an infected *Polygyra thyroides*, and then returned to a terrarium. The snails became infected and some metacercariae were approximately mature in 3 weeks and most of the metacercariae were mature in 5 weeks. The largest number of larvae recovered from any one snail was 87. The findings were verified by another experiment in which 8 snails were used and all except one became infected.

Slugs, *Deroceras laeve*, were hatched and raised in the laboratory away from the parent stock; the latter was secured in the vicinity of Beltsville, Md. When the brood of slugs was half grown, it was placed together with an infected *P. thyroides* for 24 hours as previously described. All of the slugs became infected and in a few days a few of them were killed by enormous numbers of larvae in the kidney. In the slugs which survived, the majority of metacercariae matured in 3 weeks; 60 was the largest number taken from a single specimen of the now fully grown slugs. These results were verified by a subsequent experiment in which 18 slugs were infected.

Snails, *Pseudosuccinea columella*, from a laboratory stock which had been propagated for several years, were subjected to infection by putting them with an infected *P. thyroides* for about 2 hours in a covered fingerbowl; the snails were then returned to the aquarium. Fifteen days later, a larva, now a metacercariae, was recovered from one of the snails. Snails examined subsequently were found to contain further developed cercariae and metacercariae which had completed their growth. The cercariae were found to grow at about the same rate as in other species of snails. A peculiarity was noted in the transition from cercariae to metacercariae. In a previous paper (Krull, 1935, loc. cit.) it was reported that in the growth of the cercaria the tail became increasingly more distinct and the line of demarcation between the tail and the body became more pronounced before the tail was finally discarded. This is precisely what was noted in cercariae from the snails in the present experiment, except that the entire sequence of changes seemed to be intensified and, curiously enough, in many cases the tails remained attached either partially or wholly to the bodies of the cercariae. In numerous instances the tails, which remained more or less completely attached, increased to 3 or 4 times their normal size, thereby giving the larva a peculiar appearance. In these cases the waste products from the bladder were discharged, apparently, through pores that developed at the junction of the body and tail. This retention of the tail was noted to a lesser degree in cercariae obtained from experimental infections of *Helix pomatia*.

Helisoma trivolvis and *Succinea* sp. were infected by the writer with cercariae, and the latter seemed to grow normally; none of these snails was kept alive until metacercariae had developed.

In all the infection experiments thus far described, the second intermediate host was infected by allowing it to come in contact with a first intermediate host snail. After it was discovered that aquatic snails could be infected outside of water, the question arose as to whether these snails could ever become infected in water under natural conditions. The following experiment was undertaken to settle this point.

Numerous cercariae were washed off the body of an infected *P. thyroides* into a stender dish filled with water. After a short period of activity the cercariae rolled up and formed small spheres, as previously described by Krull (1935, loc. cit.). Specimens of *Pseudosuccinea columella* were then placed in the container and left for 2 hours before removing them to an aquarium. Most of these snails became infected, and subsequent examination of specimens revealed cercariae or metacercariae, or both, depending upon the elapsed time between infection and examination.

Small snails, *P. columella,* in water, in the presence of cercariae, were studied under the dissecting microscope. It was observed that although the cercariae appeared to be inactive and, apparently, slightly swollen as a result of imbibition of water, they were not incapacitated. When the snails approached or came in contact with the cercariae, the latter were promptly stimulated and activated. They very quickly straightened themselves and by means of their suckers quickly attached themselves to the snails and began to move actively around on the body of these mollusks. It was observed also that after a sojourn on the snail many of the cercariae left the body and returned to the substratum. These cercariae resumed their locomotion for a short time, continued their measuring-worm-like movements, and when they approached or came in contact with other inactive cercariae the latter also were stimulated to activity for a short time; this response is due apparently to a chemical stimulus.

SUMMARY

The above experiments add *Helix pomatia, Deroceras laeve* and *Pseudosuccinea columella* as second intermediate host snails of *Brachylaemus virginiana.* Furthermore, it is indicated that species of the genus *Helisoma* and *Succinea* possibly may serve as second intermediate hosts of this parasite. It has been shown that a natural transfer of the cercariae from one host to another may be effected in water instead of in air, the usual medium in which the snails contact and make possible a transfer of cercariae from one snail to another; the presence of the first intermediate host is not necessary at the time the second intermediate host becomes infected since cercariae will remain alive and infective in the water for several hours. It is assumed that the response of the cercariae in becoming activated in the presence of aquatic snails, while in a water medium, is due to a chemical secreted by the snail.

Additional second intermediate hosts for *Gorgodera amplicava* Looss, 1899 (Trematoda: Gorgoderidae). Wendell H. Krull, U. S. Bureau of Animal Industry.

Since the publication of a paper by Krull (1935, Mich. Acad. Sci., Arts and Letters, 20:697-710) on the life history of the frog bladder fluke, *Gorgodera amplicava,* in which *Helisoma antrosa* was reported as a second intermediate host, the following additional snails have been determined as experimental second intermediate hosts: *Physa halei, Lymnaea traskii, Helisoma trivolvis* and *Pseudosuccinea columella.* All of the snails used in the experiment were hatched and raised in the laboratory and more than one of each became infected when exposed to cercariae by putting the snails for several days with naturally infected clams, *Musculium partumeium.* Large numbers of cysts were recovered from all of the snails except *H. trivolvis.* The largest number from *H. trivolvis* was 6 cysts, from *Physa halei* 64, from *L. traskii* 67, and from *Pseudosuccinea columella* 114. Since snails from several widely separated genera have been shown to be experimental second intermediate hosts, it may be assumed that the majority of pond snails will serve in this capacity.

Parasitic worms of equines in Panama. A. O. Foster, Gorgas Memorial Laboratory, Panama, R. de P.

Since July, 1934, the author has been privileged to study at necropsy the worm infestations of some 105 native equines, including 84 horses, 19 mules, and 2 burros. There are recorded in this paper the helminths encountered, their usual localizations in the host and data on their relative abundance. These species listed are reported for the first time from native equines of Panama.

CESTODA

1. *Anoplocephala perfoliata* (Goeze, 1782)	cecum	common
2. *A magna* (Abildgaard, 1789)	small intestine	rare
3. *Paranoplocephala mamillana* (Mehlis, 1831)	small intestine	rare

NEMATODA

4. *Strongylus equinus* Mueller, 1780	cecum	common
5. *S. edentatus* (Looss, 1900)	ventral colon	common
6. *S. vulgaris* (Looss, 1900)	cecum	very common
7. *Triodontophorus serratus* (Looss, 1900)	ventral colon	rare
8. *T. minor* (Looss, 1900)	ventral colon	common
9. *T. tenuicollis* Boulenger, 1916	ventral colon	rare
10. *T. brevicauda* Boulenger, 1916	ventral colon	rare
11. *Craterostomum mucronatum* (Ihle, 1920)	dorsal colon	very rare
12. *Oesophagodontus robustus* (Giles, 1892)	dorsal colon	very rare
13. *Gyalocephalus capitatus* Looss, 1900	ventral colon	rare
14. *Poteriostomum imparidentatum* Quiel, 1919	dorsal colon	rare
15. *P. ratzii* (Kotlán, 1919)	dorsal colon	rare
16. *Cyathostomum coronatum* Looss, 1900	cecum	common
17. *C. labratum* Looss, 1900	ventral colon	common
18. *C. labiatum* (Looss, 1901)	ventral colon	common
19. *Cylicocercus catinatus* (Looss, 1900)	ventral colon	very common
20. *C. goldi* (Boulenger, 1917)	dorsal colon	common
21. *C. pateratus* (Yorke and Macfie, 1919)	ventral colon	common
22. *Cylicostephanus calicatus* (Looss, 1900)	ventral colon	very common
23. *C. poculatus* (Looss, 1900)	cecum	rare
24. *C. longibursatus* (Yorke and Macfie, 1918)	dorsal colon	very common
25. *C. minutus* (Yorke and Macfie, 1918)	ventral colon	very common
26. *C. hybridus* (Kotlán, 1920)	ventral colon	rare
27. *C. asymetricus* (Theiler, 1923)	dorsal colon	very rare
28. *Cylicocyclus radiatus* (Looss, 1900)	ventral colon	rare
29. *C. elongatus* (Looss, 1900)	cecum	common
30. *C. nassatus* (Looss, 1900)	ventral colon	very common
31. *C. insigne* (Boulenger, 1917)	dorsal colon	very common
32. *C. leptostomus* (Kotlán, 1920)	ventral colon	common
33. *Cylicodontophorus bicoronatus* (Looss, 1900)	ventral colon	common
34. *C. mettami* (Leiper, 1913)	ventral colon	very rare
35. *C. euproctus* (Boulenger, 1917)	dorsal colon	rare
36. *C. ultrajectinus* (Ihle, 1920)	dorsal colon	common
37. *Cylicobrachytus brevicapsulatus* (Ihle, 1920)	dorsal colon	very rare
38. *Parascaris equorum* (Goeze, 1782)	upper ileum	common
39. *Oxyuris equi* (Schrank, 1788)	dorsal and small colon	common
40. *Probstmayria vivipara* (Probstmayr, 1865)	ventral colon	very common
41. *Habronema megastoma* (Rudolphi, 1819)	tumorous abscesses of cardiac glandular mucosa of stomach	rare

42. *H. muscae* (Carter, 1861)	glandular mucosa of stomach	common
43. *H. microstoma* (Schneider, 1866)	glandular mucosa of stomach	common
44. *Setaria equina* (Abildgaard, 1789)	posterior body cavity	common

Bots have been found only in animals in Chiriqui Province. These larval insects were first reported by Dunn (1934, Psyche, 41 (3): 176) who recovered 58 specimens of *Gastrophilus nasalis* from the stomach of a horse at Progreso. During a recent expedition to that region, the author found bots in 5 out of 7 mules at necropsy. The numbers varied from 2 to 21 per host, and these larval insects were located either in the pyloric region of the stomach, or in the duodenum. These larvae have been identified as *Gastrophilus nasalis* and *G. haemorrhoidalis*, the latter being reported here for the first time from Panama.

Lesions of the so called "dhobie itch," of which a nematode larva appears to be the etiological agent, are of frequent occurrence among equines in Panama.

The author wishes to acknowledge his indebtedness to Dr. H. C. Clark, Director of the Gorgas Memorial Laboratory, who personally handled many of the necropsies, to Dr. L. E. Rozeboom, Medical Entomologist to this laboratory, for identifications of the botfly larvae, and to Dr. Benjamin Schwartz, of the Zoological Division, Bureau of Animal Industry, U. S. Department of Agriculture, for valuable advice and suggestions.

Some observations on the emission of cercariae of *Schistosoma mansoni* (Trematoda: Schistosomatidae) from *Australorbis glabratus*. Arnaldo Giovannola, Rome, Italy. (Contribution from the Puerto Rico School of Tropical Medicine.)

Faust and Hoffman (1934, Puerto Rico J. Pub. Health and Trop. Med., 10:1-97) in a publication of their studies on schistosomiasis in Puerto Rico showed that the cercariae of *S. mansoni* emerge from the infected snail almost exclusively during the period from 9 A. M. to 2 P. M., confirming for this species the existence of a daily periodicity as was previously shown by Cort (1922, J. Parasitol., 8:177-183) in certain cercariae of non-human species. Faust and Hoffman stated that this phenomenon was particularly evident when the snails were exposed to direct sunlight. They regarded the emission of cercariae as chiefly due to the direct action of the light as was pointed out several years ago by Sonsino (1892, Festchr. A. 70 Geb. R. Leuckart, pp. 134-146) for the "pigmented cercariae of Amphistomum" from *Physa alexandrina* and *P. micropleura*.

On the other hand Dubois (1929, Les cercaires de la région de Neuchâtel, Thèse Méd., Univ. Neuchâtel, 177 pp.) studying the periodicity of the emission of cercariae from their snail hosts stated that the light has nothing to do with this periodical phenomenon and that the principal factor to be considered is the temperature. According to this author the temperature has not a specific action on the escape of the cercariae, but has its periodic and daily action on all stages of development of larval trematodes in the snails. During the day the higher temperature produces a decrease of the time required for the development of the germ cells which multiply very rapidly. During the night, on the contrary, the lower temperature produces a slackening of the maturation of all stages of the larval trematodes parasitic in the snails. This would show its effect in a periodic emergence of cercariae.

It seemed interesting to study the emission of cercariae of *S. mansoni* from snails kept at a constant temperature in order to establish the action of light under conditions where the daily changes of temperature were eliminated.

In this note is reported the study of the emission of cercariae of *S. mansoni* from 5 specimens of *Australorbis glabratus* during a period of 10 days. All 5 snails were infected in the laboratory and then experiments started about one month after infection. Three snails were maintained in an incubator at 31.5° C. provided with a glass door so that the light within would vary directly with the daylight. The other 2 snails were maintained as controls in the open room where both light and temperature varied. The cercariae which emerged were counted twice each day dividing the 24 hours into a period of 6 hours from 9:30 A. M. to 3:30 P. M. and a period of 18 hours from 3:30 P. M. to 9:30 A. M.

When the cercariae were to be counted the snails were removed to new bottles and a small amount of a 5% iodine solution in 70% alcohol was added to the original bottles in order to kill and stain the cercariae present. A direct count was then made by pouring the water into watch glasses.

The results obtained are reported in tables 1 and 2. It will be seen that the cercariae showed about the same periodicity in their escape from snails in the constant temperature incubator as from those in the open room. In the former case a total of 85% and the latter case 86% of all the cercariae escaped during the period from 9:30 A. M. to 3:30 P. M.

In conclusion the greatest emission of cercariae of *S. mansoni* from *Australorbis glabratus* is observed in the period from 9:30 A. M. to 3:30 P. M. each day and seems to depend chiefly on the direct action of light.

TABLE 1.—*Numbers of cercariae emerging daily from 5 snails. Experiment started January 12, 1936.*

	——Snails in open room——				Snails in constant temperature incubator (31.5° C.)					
	Snail No. 1		Snail No. 2		Snail No. 3		Snail No. 4		Snail No.5	
Days from beginning of exp.	3:30 P. M. to 9:30 A. M.	9:30 A. M. to 3:30 P. M.	3:30 P. M. to 9:30 A. M.	9:30 A. M. to 3:30 P. M.	3:30 P. M. to 9:30 A. M.	9:30 A. M. to 3:30 P. M.	3:30 P. M. to 9:30 A. M.	9:30 A. M. to 3:30 P. M.	3:30 P. M. to 9:30 A. M.	9:30 A. M. to 3:30 P. M.
1	22	654	25	761	146	407	42	88	140	243
2	8	986	12	1,404	44	1,311	8	35	149	599
3	415	1,585	209	870	128	1,831	19	291	359	1,985
4	30	1,528	42	1,022	186	2,349	15	438	264	5,721
5	148	883	220	1,097	946	1,695	37	589	320	995
6	304	1,795	849	2,167	27	389	152	840	557	2,939
7	.....	.....	——dead——		....	.....	....	.....	.....	.....
10	89	1,925	....	.....	10	278	12	382	31	306

TABLE 2.—*Summary of the number of cercariae emerging from 5 snails (for numbers of cercariae emerging each day see Table 1).*

	Snails Nos. 1 and 2 in ——open room——		Snails Nos. 3, 4, 5 in constant temperature incubator (31.5° C.)	
	3:30 P. M. to 9:30 A. M.	9:30 A.M. to 3:30 P. M.	3:30 P. M. to 9:30 A. M.	9:30 A. M. to 3:30 P. M.
Total number	2,369	16,676	3,592	23,711
Daily ave. number for each snail....	182	1,283	171	1,130
Percentage	14	86	15	85

Miracidial twinning in *Schistosoma mansoni* (Trematoda: Schistosomatidae). W. A. Hoffman and J. L. Janer, School of Tropical Medicine, San Juan, Puerto Rico.

During the past year while making examinations of the excreta of monkeys harboring *Schistosoma mansoni* 2 pairs of twin miracidia were encountered. In both instances the double organisms were fused anteriorly for approximately one-third of their length. The first pair noted pursued a more or less circular path, possibly because one of the members possessed greater strngth or ease of movement. When observed in motion this pair bore some slight resemblance to the rostellar hook of a cestode. The other example apparently swam with little difficulty, both members seeming to coordinate quite readily in developing a smooth forward movement. This pair was placed in close apposition to a mature specimen of *Australorbis glabratus* (*Planorbis guadeloupensis*), the intermediate host of *Schistosoma mansoni.* Though the paired miracidia came into contact with the snail quite frequently during a period exceeding 15 minutes they paid no attention to it. Later, the same snail was exposed to several normal miracidia, some of which penetrated in the normal manner. Failure of these miracidial twins to enter could therefore not be ascribed to unsuitability on the part of the host species. The 2 abovementioned specimens were encountered in the excreta of different monkeys.

An abnormal ovary in *Fasciola hepatica* (Trematoda: Fasciolidae). W. A. Hoffman, School of Tropical Medicine, San Juan, Puerto Rico.

References pertaining to structural abnormalities of cestodes occur in the literature with relative frequency while those concerning abnormalities in trematodes have been recorded rarely. Recently after staining a specimen of *Fasciola hepatica* obtained from the liver of a cow at a local slaughter house, an examination of the worm disclosed a condition of bilateral ovary. Just beyond the ootype dichotomous branching of the ovary occurs (fig. 17), one division leading to the right side of the body, where the ovary is normally found, the other to the left. The latter subdivides to some extent, though not to the same degree as on the normal side. The right side likewise seems to possess fewer branches than are usually observed in that organ. While cases of bilateral ovary in *Fasciola hepatica* are not unknown, such cases are rare and warrant being placed on record.

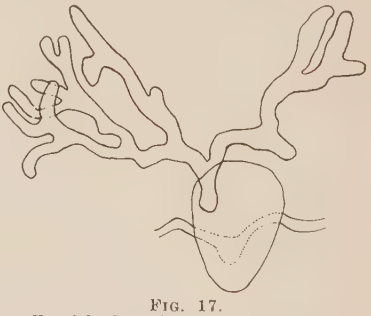

Fig. 17.
Fasciola hepatica, abnormal ovary.

New records on the prevalence and distribution of some Telorchiinae from *Pseudemys elegans* Wied. H. J. Bennett and J. E. Tobie, Louisiana State University.

In the fall of 1935 an examination was made of 33 specimens of the turtle host, *Pseudemys elegans,* from the vicinity of Baton Rouge, Louisiana. The digestive tract, liver, lungs, and bladder were examined in every host with complete records kept on the trematodes, nematodes, and Acanthocephala. The Telorchiinae used in this note were recovered from the small intestine.

There were recovered from 15 of the 33 hosts 275 trematodes, 236 of which belonged to the Telorchiinae of the genera *Protenes* and *Cercorchis.* Three species of *Cercorchis* were found, *Cercorchis corti* (Stunkard, 1915) Perkins, 1928, *Cercorchis singularis* Bennett, 1935, and *Cercorchis medius* (Stunkard,

1915) Perkins, 1928. In 1900 Luhe created the genus *Cercorchis* which was not accepted by Stunkard 1915. Perkins 1928 redefined *Cercorchis* and raised it to generic rank. In this paper *Cercorchis* Luhe, 1900 as redefined by Perkins, 1928 is considered as being valid. *C. corti* has by far the greatest prevalence and distribution consisting of 213 of the 236 trematodes recovered and being found in 10 hosts. The numbers in the individual host ranged from 2 to 67. *C. singularis* was recovered in much smaller numbers, being found in only 2 hosts, 6 in one host and 1 in the other. *C. medius* was the least prevalent, there being only 3 specimens found, 1 in each of 3 hosts.

Protenes vitellosus Bennett, 1935, was found in only 2 hosts, there being 10 in one and 3 in the other.

In the spring of 1936 further examinations were made of the host *P. elegans*. *C. singularis* showed a much higher degree of infestation than in the hosts examined in the fall of 1935. There were as many as 58 specimens recovered from a single host.

On the assignment of *Echinorhynchus dirus* to the genus *Acanthocephalus*. Harley J. Van Cleave and Lee H. Townsend (Contributions from the Zoological Laboratory of the University of Illinois, No. 486).

Echinorhynchus dirus Van Cleave, 1931, was described (Trans. Amer. Micros. Soc., 50:348-363) from the freshwater drum (*Aplodinotus grunniens*) of the Yazoo River at Money, Mississippi. Since the date of the original description of this species new records of its occurrence have come to our attention. Some of the material in these more recent accessions is in better histological condition than that of the original lot. A restudy of all available specimens has given proof that this species belongs to the genus *Acanthocephalus* Koelreuther and should be designated as *Acanthocephalus dirus* (Van Cleave, 1931). The brain and retinacula are particularly prominent in some of the recently studied material and have the distinctive position at the posterior extremity of the receptacle as diagnostic for the genus *Acanthocephalus*. In many of the specimens the lemnisci are considerably longer than "two-thirds the length of the receptacle" as given in the original description. In a few preserved specimens they are as much as one-fourth longer than the receptacle and very often are about the same length as that organ.

The transfer of this species to the genus *Acanthocephalus* becomes the first instance of a member of this genus to be recorded from North American fishes. The entire genus is very poorly represented in the North American fauna. Two reports of the occurrence in amphibians compose the only previous instances of *Acanthocephalus* in North American hosts.

In addition to the type locality in the lower Mississippi River drainage, records are now available for the occurrence of *Acanthocephalus dirus* in the type host (*Aplodinotus grunniens*) from the Ohio River at Shawneetown, Illinois, and from the Grand Pierre River at Rosiclare, Illinois. That the species has become firmly established in the Ohio River drainage is evidenced by the fact that it has been taken in the bluegill (*Helioperca incisor*) and in the channel cat (*Ictalurus punctatus*) in addition to its type host.

In the years from 1909 to 1921 the senior author of this note periodically made general surveys of the acanthocephalan fauna of fishes of the Illinois River in the vicinity of Havana and of the upper Mississippi River at various stations, particularly at Fairport, Iowa, and at Lake City, Minnesota. In 1921 when these periodic surveys were discontinued no instance of the occurrence of *Acanthocephalus dirus* in the Illinois or the upper Mississippi had been encountered. In 1933 and subsequently various fishes of the Illinois River and tributary streams in the vicinity of Havana have been found heavily parasitized with *Acanthocephalus dirus*. One of the heaviest infestations encountered here was in a large mouth black bass (*Huro salmoides*). The recent appearance of this species in fair abundance at Havana indicates that the species is actively spreading its geographical range within recent years.

A method for obtaining adults of *Stephanofilaria stilesi* (Nematoda: Stephanofilariidae). G. Dikmans, U. S. Bureau of Animal Industry.

While a diagnosis of stephanofilarosis in cattle can be made with reasonable certainty on the basis of the location and clinical appearance of the lesions and while such a diagnosis can at times be confirmed by the finding of the embryos of *Stephanofilaria* inclosed in a vitelline membrane, in the scrapings from such lesions, it has been rather difficult to obtain adults of *Stephanofilaria* from skin lesions submitted for examination. Recently, in the course of an examination of a number of lesions obtained from cattle in one of the abattoirs in Baltimore, it was found that adults of *Stephanofilaria* could be obtained in fair numbers with relative ease by the following method.

Pieces of skin showing characteristic lesions were sliced into one-quarter to one-half inch slices and the slices were ground in an ordinary No. 1 meat grinder. The ground up material was placed in a Baermann apparatus with water or physiologic saline at a temperature of 48° to 50° C. The material was left in the apparatus overnight at room temperature or placed in a room in which the temperature was maintained at 37.5° C. The next morning the liquid in the stem of the funnel was drawn off and centrifuged. The residue was examined and found to contain embryos, larvae, and intact adult males of *Stephanofilaria*. No intact adult females were found in this residue; however, the presence of embryos in the residue indicated the presence of females. The ground up material was placed in a coarse screen, washed, and the washings concentrated and examined for females. Intact adult females of *Stephanofilaria* were found in these washings. An examination of these specimens showed that in most cases the posterior two-thirds of the body was rolled in a loose spiral; this may be the reason why the females did not pass through the fine meshes of the screen on which the ground up portions of skin were placed.

The springbuck, *Antidorcas marsupialis*, a new host of the lungworm, *Bronchonema magna* Mönnig, 1932 (Nematoda: Metastrongylidae). G. Dikmans, U. S. Bureau of Animal Industry.

The lungworm, *Bronchonema magna* Mönnig, 1932, was reported as having been collected from the blesbuck, *Damaliscus albifrons*, at Onderstepoort, Union of South Africa. So far as available records indicate, it has not been reported from any other animal. In connection with a recent study of lungworms of cattle and deer, some nematodes collected from the lungs of a springbuck, *Antidorcas marsupialis*, at the National Zoological Park, Washington, D. C., which had been labelled *Dictyocaulus*, were examined. It was found that these nematodes did not belong in the genus *Dictyocaulus* but in the genus *Bronchonema*. Mönnig, however, describes and figures a distinct gubernaculum, 200μ long, as being present in *Bronchonema magna*, while in the male specimens of *Bronchonema* from *Antidorcas marsupialis* no such structure could be observed. There appeared to be an extremely lightly cuticularized, slightly granular body in the place where one would expect to find the gubernaculum but the writer cannot interpret this body as a gubernaculum. However, because of the small number of male specimens available for examination and because these lungworms from *Antidorcas marsupialis* are otherwise identical with *Bronchonema magna* from *Damaliscus albifrons*, as described and figured by Mönnig, and further because these nematodes were collected from closely related hosts, the writer tentatively identifies the specimens from *Antidorcas marsupialis* as *B. magna* pending an opportunity for examining a larger number of specimens.

A method for recovering the strongyle larvae of the horse. H. L. VAN VOLKENBERG, Puerto Rico Experiment Station, U. S. Department of Agriculture, Mayaguez, Puerto Rico.

Attempts to obtain large numbers of infective larvae of the large and small strongyles of the horse by the ordinary charcoal-culture method have been unsatisfactory. The sieving of the bulky manure requires considerable time and labor, and only a small percentage of the larvae have been recovered on passing the culture material through the Baermann apparatus. A more simple and direct method for recovering these larvae has been found.

The fresh manure in the form of balls is collected and stored in a place inaccessible to fly oviposition. The ensheathed, infective larvae have been recovered within 4 days. The 4-day-old or older balls of manure are broken up and placed directly in a Baermann apparatus. Water drawn directly from the tap is used in the apparatus. By employing a 40 mesh to the inch sieve, 8 inches in diameter and 2 inches in depth, and by changing the manure 4 times at 3-hour intervals, approximately 9,000 larvae have been recovered from manure of a heavily infested animal.

The larvae can be retained in an inactive state for a month or more with little loss in numbers. After drawing from the Baermann apparatus and centrifuging, the liquid containing the larvae is allowed to evaporate at room temperature. The dish containing the larvae is stored in a chamber having a high humidity. The larvae can be revived by adding water. They will survive and remain active for 10 days or more in a shallow dish of water.

One disadvantage of this method has been found; often the more heavily infested animals are suffering apparently from a catarrhal enteritis as evidenced by considerable mucus in the feces. This mucus or mucoid substance passes through the Baerman apparatus and the larvae become entangled in it. The entangled larvae do not settle on standing, or by centrifuging, and are lost.

A note on the use of brilliant green as an anthelmintic for chickens. W. H. WRIGHT, U. S. Bureau of Animal Industry (Transferred to National Institute of Health), and H. L. VAN VOLKENBERG, Puerto Rico Agricultural Experiment Station, Mayaguez, P. R.

In a series of tests carried out at the Puerto Rico Agricultural Experiment Station with a number of dyes of varied chemical constitution, brilliant green (tetraethyl-diamino-triphenyl-methane-sulphate) was administered in hard gelatin capsules in doses of 5 to 15 grains to 8 chickens weighing between 1.45 and 4.75 pounds. The compound proved markedly toxic to the birds in question and 5 of them died as a result of the treatment. The efficacy of the treatment against *Ascaridia galli* varied between 10 and 100 per cent. The drug apparently removed all specimens of *Raillietina tetragona* and an undetermined species of *Raillietina* from all of the experimental birds infested with these tapeworms; it was ineffective against *Capillaria annulata, Tetrameres americana, Cheilospirura hamulosa, Strongyloides avium, Capillaria retusa, Heterakis gallinae, Postharmostomum* sp. and the smaller tapeworms—*Davainea proglottina, Amoebotaenia sphenoides* and *Hymenolepis cantaniana.* All parts of the tapeworms, including the heads, removed by the dye were deeply stained. The birds used in this test were in very poor condition and the doses of the drug employed were relatively large. The smallest dose, 5 grains, was as effective for the removal of *Raillietina* spp. as were the larger doses. While brilliant green proved to be very toxic to the chickens in question, it is possible that further work may result in the finding of an effective, non-toxic dose rate, or it may be possible to combine the dye with some inert substance which would reduce the rate of absorption of the compound from the digestive tract of the bird and thus reduce the toxicity. Time was not available to carry out additional tests but the results of the present test would seem to indicate that this compound, and possibly other dyes of allied chemical structure, may be of value for the removal at least of some species of tapeworms from poultry.

Notes on the spread, in one year, of helminths from infected to uninfected poultry yards. MARGERY W. HORSFALL, U. S. Bureau of Animal Industry.

On April 18, 1935, 30 laboratory-raised Rhode Island chickens, 10 months old, were placed in a newly fenced, uninfected experimental poultry yard, at the Zoological Division field station at the National Agricultural Research Center, Beltsville, Maryland. These chickens had been used in an experiment on coccidiosis and had recovered from this infection. Droppings, which had been examined frequently while the birds were caged, were consistently negative for helminth eggs and coccidia oocysts. The experimental yard in which these birds were placed was 140 by 110 feet, and was located on a well drained, partially shaded, west slope; as far as is known to the writer, no chickens had previously ranged over the area in question. The nearest poultry yards which contained chickens infested with parasitic worms were approximately 300 feet away and a little higher on the same slope. A small creek intervened between the poultry yards and the experimental plot, thus eliminating drainage from the infected pens to the experimental plot.

From April 18, 1935, to April 18, 1936, chickens from the experimental plot and from the infected pens were frequently examined post mortem, and droppings were examined microscopically for worm eggs and macroscopically for tapeworm segments. The following species of nematodes and cestodes, arranged in the order of their abundance, were found in the chickens from the infected pens: *Heterakis gallinae, Ascaridia lineata, Hymenolepis carioca, Raillietina cesticillus, R. echinobothrida, R. tetragona, H. cantaniana, Choanotaenia infundibulum* and *Davainea proglottina.*

On May 17, 1935, 29 days after the chickens had been placed in the experimental yard, each of 18 out of 24 fresh fecal samples collected at random in the plot contained 5 to 47 chains of *Hymenolepis carioca* segments. On July 10, 1935, one rooster passed a few segments of *Raillietina cesticillus.* On August 3, 1935, eggs of *Heterakis gallinae* and *Ascaridia lineata* were found in 4 out of 11 fresh fecal samples collected in the experimental plot. Post-mortem examinations of chickens from this lot at a later date verified the presence of these 4 species of helminths. No indications of infestations with other parasitic worms were found in the birds during the remainder of the year that the experimental pen was kept under observation.

The 4 helminths most common in the chickens of the infected pens were transmitted to chickens in the experimental pen, but not in the order of their relative abundance. The two tapeworms, *Hymenolepis carioca* and *Raillietina cesticillus,* that are carried by dung beetles and flies appeared first. *Aphodius granarius,* a common intermediate host for these cestodes was abundant in both infected and experimental yards and could be seen flying during April and May. Since specimens of this beetle containing cysticercoids of *Hymenolepis carioca* were found in the experimental yard, this intermediate host was probably a means of introducing into the experimental yard the two cestodes in question. The 2 nematodes which appeared in chickens kept in the experimental yard have direct life histories; they appeared in the chickens considerably later than the cestodes. It is likely that the eggs of these nematodes were carried mechanically, possibly on shoes of attendants. The other species of worms found in the chickens of the infected pens utilize beetles, flies, snails and ants as intermediate hosts. The latter species of helminths have not been found in the experimental pen up to the present time. There are other infected poultry yards about one quarter mile away, but their location, distance and the habits of attendants who take care of them, make it seem unlikely that the infection came from these more distant yards.

Only 4 species of worms, 2 with intermediate hosts and 2 with direct development, appeared in chickens placed in a clean yard. Their presence under the conditions of this experiment indicate that even a short distance of 300 feet from yards containing infested chickens constitutes a limited barrier to the spread of poultry helminths.

Two new trematodes from African reptiles. E. W. PRICE, U. S. Bureau of Animal Industry.

In 1926, the Smithsonian-Chrysler Expedition to East Africa secured for the National Zoological Park, Washington, D. C., a large variety of animals, including mammals, birds and reptiles. Owing to injuries incurred during shipment and also to changes in environment, there was a high mortality among these animals after their arrival at the Park. Through the courtesy of the officials of the U. S. National Museum the writer was permitted to examine the viscera of a large number of these animals for internal parasites, and as a result of this a considerable collection of trematodes, cestodes and nematodes was obtained. Several of the species collected appear to be new, and the purpose of this paper is to present descriptions of two of the new trematodes obtained from reptiles.

Family PARAMPHISTOMIDAE

Schizamphistomoides constrictus, n. sp.

Description.—Body elongate (fig. 18, A), 3 mm long by 0.68 mm wide, with slight but distinct constriction immediately anterior to acetabulum. Cuticula smooth. Rudimentary eyespots, represented by 2 scattered masses of pigment slightly posterior to level of oral pouches, present. Oral sucker elongated, 350μ long by 185μ wide, slightly retracted into anterior end of body, with 2 oral pouches, 80μ long, directed postero-dorsally; acetabulum 480μ long by 415μ wide, opening ventrally at posterior end of body. Esophagus 270μ long; esophageal bulb 240μ long by 96μ wide; intestinal ceca simple, extending to slightly beyond level of ovary. Excretory vesicle relatively small, oval, immediately postovarial; excretory aperture dorsal and median, about midway between ovary and anterior margin of acetabulum. Genital aperture at level of intestinal bifurcation. Testes globular, about 175μ in diameter, tandem, postequatorial, about 175μ apart. Ovary about 40μ long by 74μ wide, median or nearly so, slightly anterior to level of ends of intestinal ceca. Vitellaria not developed. Uterus median, slightly sinuous, in median line and passing dorsal to testes.

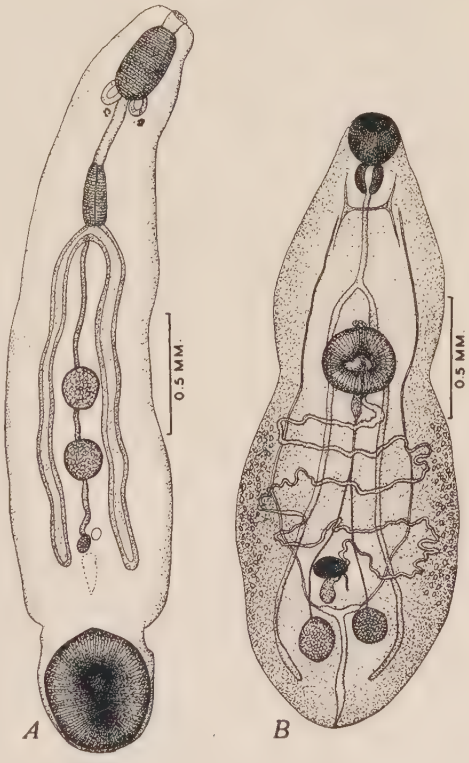

FIG. 18.
A—*Schizamphistomoides constrictus*, ventral view.
B—*Cyclorchis varani*, ventral view.

Host. — *Pelomedusa galeata* (Schoepff).

Location.—Large intestine.

Distribution.—Africa (Tanganyika Territory).

Specimen.—U. S. N. M. Helm. Coll. No. 27390 (type).

Only a single immature specimen of this species was obtained although several tortoises were examined. Despite the immaturity of the specimen, there appear to be adequate characters to distinguish this form from the other 2

species of the genus. The following key will serve to separate the species of *Schizamphistomoides*:

1. Maximum body width at level of acetabulum; testes preequatorial; from marine turtle, *Chelone mydas*.....*spinulosum* (Looss)
   Maximum body width preacetabular; testes equatorial or postequatorial; from fresh-water turtles..... 2
2. Acetabulum set off from body proper by constriction; testes postequatorial; from *Pelomedusa galeata*.....*constrictus*, n. sp.
   Acetabulum not set off from body proper by constriction; testes equatorial; from *Dermatemys mawii*.....*tabascensis* Caballero and Sokoloff

Family OPISTHORCHIIDAE

Cyclorchis varani, n. sp.

Description.—Body elongate oval (fig. 18, B), 2 to 2.2 mm long by 0.8 to 0.976 mm wide, flattened dorso ventrally, and with distinct constriction at level of acetabulum. Cuticula delicate, without spines. Oral sucker subterminal, 144 to 192μ in diameter; acetabulum 176 to 256μ in diameter, about one-third of body length from anterior end. Pharynx 80 to 120μ in diameter; esophagus 300 to 320μ long; intestinal ceca slender, frequently showing alternating dilations and constrictions, extending parallel in median field to about beginning of posterior body third, then curving laterad as far as level of testes and then mediad, finally terminating about 200μ from posterior end of body. Excretory vesicle T-shaped, with relatively long, slightly sinuous stem and short branches; collecting ducts extending anteriad in lateral fields, following a course about midway between lateral margins and intestinal ceca. Genital aperture median, immediately preacetabular. Cirrus pouch absent; seminal vesicle slender and tortuous, extending slightly beyond posterior margin of acetabulum. Testes globular, slightly unequal, usually directly opposite each other, about one-sixth of body length from posterior end; right testis 128 to 160μ in diameter, left testis 112 to 114μ in diameter. Ovary transversely oval or slightly trilobed, 80μ long by 100 to 112μ wide, pretesticular and slightly to right of median line. Seminal receptacle slightly retort-shaped, immediately postovarial. Laurer's canal slender, opening dorsally in median line at level of seminal receptacle. Vitellaria lateral, extending from a short distance posterior to level of acetabulum to about level of seminal receptacle. Uterus slender, consisting of a few transverse loops extending far into extraintestinal fields. Eggs about 30μ long by 15μ wide.

Host.—*Varanus niloticus* (Linn.).

Location.—Small intestine.

Distribution.—Africa (Tanganyika Territory).

Specimens.—U. S. N. M. Helm. Coll. No. 27448 (type and paratypes).

About 20 specimens of this species were collected from the small intestine of one of several monitors examined. The specimens were quite uniform as regards size and shape, but showed some indications of senility as evidenced by the presence of but few eggs, some being malformed, and scattered irregularly throughout the course of the uterus; the reproductive organs, particularly the vitellaria showed evidence of atrophy.

Cyclorchis varani may easily be distinguished from both *C. amphileucus* (Looss) and *C. campula* (Cobbold) by the distinct constriction at the level of the acetabulum in the former species, whereas in the latter 2 species no such constriction exists. Other differences exist and the more outstanding of these are brought out in the following key:

1. Proximal portion of uterus passing between ovary and testes; from cetacean, *Platanista gangetica*.....*campula* (Cobbold)
   Proximal portion of uterus not passing between ovary and testes; from reptiles 2
2. Acetabulum about one-fourth of body length from anterior end; testes distinctly diagonal in position; from *Naja haje*.....*amphileucus* (Looss)
   Acetabulum about one-third of body length from anterior end; testes opposite each other, or nearly so; from *Varanus niloticus*.....*varani*, n. sp.

Notes on the strawberry strains of the bud and leaf nematode, *Aphelenchoides fragariae*, I. J. R. Christie and Louise Crossman, U. S. Bureau of Plant Industry.

(1) *The experimental infestation of strawberry plants.*—In connection with investigations of the strawberry strains of *Aphelenchoides fragariae* (Ritzema Bos, 1891), it is frequently useful to have a reliable method by which strawberry plants can be experimentally infested. The following experiments were an attempt to test the effectiveness of suspending the nematodes in water and placing them in the crown of the plant with a dropper.

In the first experiment a drop of water was placed near the bud between the small unfolding leaves and the nematodes transferred to this drop with a bamboo pick. This was, in effect, the same as transferring the specimens directly to the plant in a drop of water except that it permitted more accurate determination of the number of specimens used. In the case of 2 plants, small pieces of an infested bud were placed on the plant in a drop of water. A female was selected for the plants that received 1 specimen, otherwise the sex was not determined. The infestations were made on October 8, 1932, and the plants were then placed in the greenhouse. On the bench beside the plants thus treated were 16 uninfested plants to serve as controls. From 151 to 164 days later all plants were examined carefully and all specimens of *A. fragariae* counted; none of the plants had developed perceptible symptoms of dwarf. The results are shown in table 1.

Table 1—*Results of infestation experiment.*

No. specimens used	No. specimens found	No. specimens used	No. specimens found
1	57	10	27
1	74	10	105
1	2	10	50
1	78	10	0
1	0	10	2
1	53	10	120
10	4	piece of bud	73
10	243	piece of bud	167

This experiment indicates that under greenhouse conditions placing the parasites in a drop of water at the crown of the plant near the bases of the leaf petioles is usually effective in establishing an infestation. Failure of a plant to develop symptoms is not necessarily an indication that an infestation was not established. This is especially true under greenhouse conditions.

At the North Carolina Coastal Plain Station, Willard, N. C., a cement isolation frame was constructed, the walls of which extend 18 inches below soil level and 18 inches above and inclose an area 12 feet square. In this area were set 64 strawberry plants of the Blakemore variety, arranged in 8 rows of 8 plants each.

On July 18, 1935, buds were secured from a large number of locally obtained strawberry plants infested with *A. fragariae*. These were triturated in water and allowed to stand 30 minutes. The water was then drawn from the plant fragments and centrifuged. About two-thirds of the supernatant fluid was drawn from the centrifuge tubes, filtered through paper and placed in flask A. The remainder of the fluid in the tubes, which contained the nematodes, was agitated and poured into flask B.

By means of a fine pointed dropper the contents of the flasks were dropped into the crowns of the plants in the isolation frame. The fluid was placed around the bud at the base of the leaf petioles. Alternate rows received the contents of flask A and intervening rows the contents of flask B. The operation was performed during late afternoon and the sky was partly overcast. The plants were not covered.

On August 13, none of the plants showed noticeable symptoms of dwarf. On September 14, 20 of the plants receiving the contents of flask B (containing the nematodes) showed unmistakable symptoms of dwarf, 2 showed probable symptoms, 9 did not show recognizable symptoms and 1 was missing. Of the 32 plants receiving the contents of flask A none showed recognizable symptoms of dwarf. The plants were not examined microscopically.

The experiment was primarily for the purpose of testing this method of infesting strawberry plants with *A. fragariae* under field conditions at Willard. Results are in agreement with those obtained by Brooks (1931, Fla. Agr. Expt. Sta. Bull. 235).

(2) *Rearing the strawberry strain of the bud and leaf nematode on culture media.*—Christie and Arndt, in a paper to appear in the July, 1936, issue of Phytopathology, will report the successful rearing of *Aphelenchoides parietinus* (Bastian, 1865) on agar cultures. The medium used was the same as that given herein and the cultures were inoculated with a suitable fungus from which the nematodes derive their food by inserting the stylet and removing the contents of hyphae. The present writers have succeeded in rearing *A. fragariae* from North Carolina strawberry plants in a similar manner. Not all fungi are suitable for rearing this species. Best results have been obtained with *Alternaria citri*, but doubtless there are many others which will serve as well. Cultures of *A. fragariae* from North Carolina strawberry plants have been continuously maintained in this manner for a period of about 8 months. They have built up large populations and furnish ample material for experimental purposes.

A. fragariae from Massachusetts strawberry plants have been continuously reared on similar cultures for a period of about 6 months. Much more difficulty has been experienced in rearing specimens from this source. They continue to survive and reproduce sufficiently to maintain themselves but have never built up large populations. This appears to be further evidence of the existence of 2 strains of *A. fragariae* from strawberry plants.

Attempts to rear *A. fragariae* from chrysanthemums (*A. ritzema-bosi* of authors) have failed.

The successful rearing of *A. fragariae* from strawberry plants appears to require the following conditions: (a) Presence of a suitable fungus, (b) a comparatively dry culture with only a thin film of moisture over the surface of the agar and (c) absence of bacteria. The agar medium used by the writers was prepared as follows:

A. Extract 15 grams of cornmeal in 750 cc of water and filter.
Add water to filtrate to make 500 cc.
Add 2.5 grams malt extract.
7.5 grams sucrose.
20 grams agar.

B. 500 cc plant nutrient solution.
1 cc concentrated lactic acid.

Any standard plant nutrient solution will serve the purpose. The one used by the writers was made by adding the following salts to a liter of water: KH_2PO_4, 2.45 grams; $Ca(NO_3)_2$, 0.85 grams; $MgSO_4$, 3.69 grams; Ferric citrate, trace.

To avoid hydrolysis autoclave A and B separately and combine just before pouring the plates. The plates were usually inoculated with the fungus 2 days before the nematodes were transferred. This procedure seems unnecessarily complicated and a simplified culture medium, equally satisfactory and easier to prepare can probably be found, although the writers' attempts to accomplish this were not so very successful.

In order to reduce to a minimum bacterial growth and fungal contaminations the nematodes were sometimes treated with a mercuric chloride solution before they were placed on the plates. This was done as follows: A small quantity of water was poured over the plate from which the nematodes were being transferred. After 5 or 10 minutes the water, together with the nematodes, was poured into a centrifuge tube and centrifuged for 2 or 3 minutes

at a moderate speed. Most of the water was then drawn off, leaving the nemas in a few drops at the bottom of the tube. A 1 to 2,000 solution of mercuric chloride was then poured into the tube and allowed to stand for 30 minutes. The specimens were again centrifuged to the bottom of the tube and the mercuric chloride solution drawn off. The nematodes were washed twice by filling the tube with sterile water, centrifuging and drawing off the supernatant fluid. They were then transferred to the new plates. Specimens from North Carolina plants withstand this treatment well, in fact the strength of the solution or the time of treatment probably could be increased if necessary. The effect of the treatment on specimens from Massachusetts plants has not been thoroughly tested but they seem to be less resistant.

(3) *Hot-water treatment of strawberry plants.*—In cooperation with the North Carolina Coastal Plain Station, Willard, N. C., an experiment with the hot-water treatment of strawberry plants was conducted between January 7, and January 11, 1936. It was primarily a tolerance test and this was selected as the time of the year when plants would be most likely to survive in this region. Plants of the Blakemore variety were used and the following treatments were given, 100 plants being treated in each case: 120° F. for 20 minutes, 115° F. for 2 hours and 15 minutes, 110° F. for 8 hours, and 106° F. for 30 hours. The plants were treated, cooled (but not plunged into cold water) and set directly into the field. Weather conditions were favorable. Heavy rain had fallen a few days previously and more rain came shortly after the plants were set.

On March 28 the following plants were making a weak growth: 120° F. for 20 minutes, 39 plants; 115° F. for 2 hours and 15 minutes, 5 plants; 110° F. for 8 hours, 10 plants; 106° F. for 30 hours, none of the plants. Eventually all of these plants died.

These treatments were based on the thermal death point of *Aphelenchoides fragariae* from southern-grown plants as noted in a previous paper (Christie and Crossman, 1935, Proc. Helminth. Soc. Wash., 2:98-103). They are the shortest treatments which can be expected to rid the plants of this nematode and even longer treatments may possibly be necessary. The results of this test make it appear exceedingly unlikely that hot water can be employed as a means of treating southern-grown strawberry plants for this pest.

(4) *The eggs of nematodes inhabiting the buds of strawberry plants.*—Nematodes other than *Aphelenchoides fragariae* are frequently found on strawberry plants. Like *A. fragariae* they are usually located at the bases of the leaf petioles or between the leaf primordia and young unfolding leaves. Of these *Cephalobus elongatus* de Man, 1880, has been found most frequently by the writers. Whenever parts of the bud have been killed, *C. elongatus* is often present in large numbers. During early spring, when dwarf symptoms are first appearing in the region of Falmouth, Mass., the nematode population of affected plants consists almost exclusively of *A. fragariae.* A few weeks later, on the other hand, fully half the nematode population of these same plants may be *C. elongatus.* In North Carolina blossom buds are frequently stimulated into growth by warm winter weather and subsequently killed by freezing. This may result in dead tissue at the crown and such plants are usually invaded by *C. elongatus.* There is no evidence that strawberry plants are appreciably injured by the presence of this nematode.

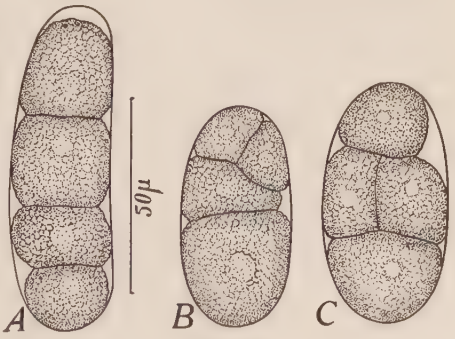

FIG. 19

Eggs of nematodes most commonly found in strawberry plants. A—*Aphelenchoides fragariae.* B—*A. parietinus.* C—*Cephalobus elongatus.*

Although the writers have only occasionally found a few specimens of *A. parietinus* in strawberry plants, the reports of other investigators indicate that it is sometimes present in considerable numbers. Occasional specimens of dorylaims, rhabditids, etc., have been encountered but never in abundance.

A. fragariae can be easily differentiated from *A. parietinus* and *C. elongatus* by the shape of the egg (fig. 19). For purposes of comparison eggs were selected which had been deposited not over 2 hours. They were of approximately the following sizes: *A. fragariae,* 68 by 21μ, or slightly over 3 times as long as wide; *A. parietinus,* 46 by 23μ or twice as long as wide; *C. elongatus,* 50 by 26μ or about twice as long as wide. In all three cases, eggs of the same species in the same stage of development vary considerably in size and to some extent in shape. In spite of these variations the diffrence between the shape of eggs of *A. fragariae* and eggs of the other two species most commonly found in the buds of strawberry plants is sufficiently distinct that one can detect it even with a dissecting microscope, or, possibly, with a hand lens.

Metal slide mounts for microscopic objects. W. D. Courtney, U. S. Bureau of Plant Industry (Sumner, Wash.).

In 1917, the late Dr. N. A. Cobb published a paper (1917, Contributions to a Science of Nematology V, pp. 123-124) in which he made reference to and gave an illustration of, the metal slide mount. In his discussion of improved microscope technique he stated, "The use of an ordinary apochromatic objective as a condenser necessitates the use of a special object slide, consisting essentially of a carrier, and two cover glasses. The object is mounted between the cover glasses."

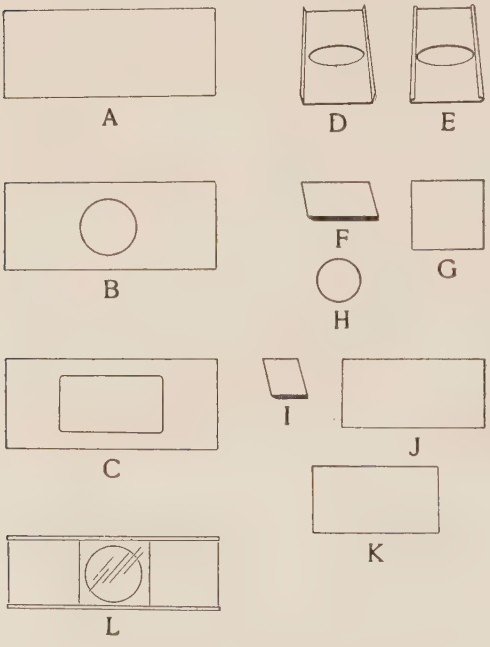

Fig. 20. Metal slides

A—Blank slide before punching and crimping. B—Punched blank slide before crimping. C—Blank slide punched with rectangular hole to permit use of larger cover-glasses. D—Slide after first crimp. E—Slide after second crimp. F—Pasteboard tabs for slide shown in B. G—Lower cover-glass, 25 mm square, for slide shown in B. H—Top cover-glass, 15 to 18 mm in diameter, for slide shown in B. I-K—Pasteboard tab, upper cover-glass and lower cover-glass, respectively, for slide shown in C.

The metal slide mount has proved useful in making it possible for the microscopist to view both sides of the mounted specimen with the higher powers of the microscope. For those investigators who wish to use apochromatic objectives as substage condensers or who desire to observe both sides of the mounted specimen, the following description will be of aid in constructing the Cobb type of metal slide mounts for laboratory use. Unfortunately these mounts are not obtainable through any of the scientific supply companies, so that each user must construct his own.

MATERIAL

Tin and aluminum have been used to make these metal mounts. Either metal in thin sheets, about 0.15

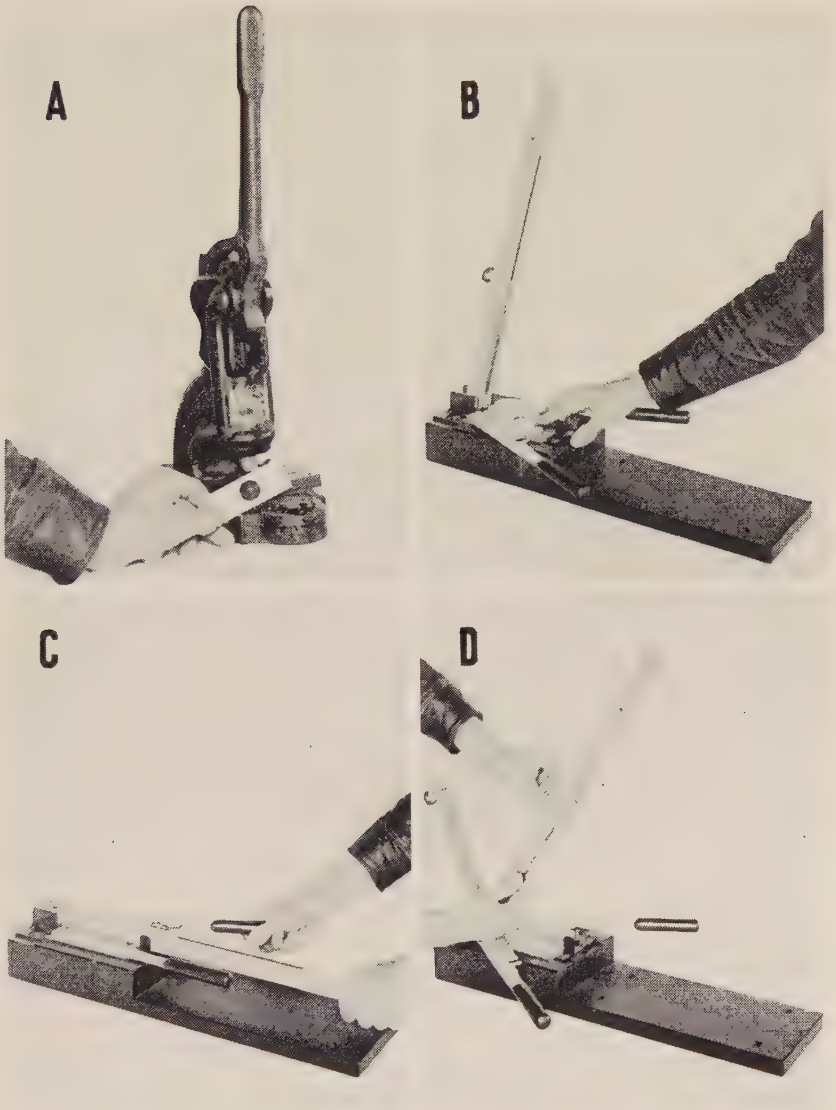

FIG. 21. Punching and crimping metal slides

A—Punch and punched slide. B—Slide former with slide in position for crimping. C—Slide former with long top-level lowered to make the first crimp and side levers being closed to make the second crimp. D—Slide former with top and side levers released; the short top lever has been moved back making it possible to compress the sides of the long top lever and slip off the crimped slide.

mm, may be cut into long strips 76.2 mm (3 in.) wide. These strips are then cut into pieces 30 mm wide, so that each resulting blank slide will be 76.2 mm (3 in.) long by 30 mm wide by 0.15 mm thick. These blank slides are then ready for the punching and crimping to be described later.

The pasteboard tabs (fig. 20, F) are made from good pasteboard stock 1.05 mm thick. This stock is cut into strips 25 mm wide. Each strip is

cut into 27 mm pieces—one end of each piece being cut on a bevel, by raising one end of the strip on the paper cutter for the beveled cut. The 27 mm measurement should include the entire length of the tab. The pasteboard stock should be of a color that will not show thumb marks and with a surface that will take ink for labeling.

The 27 mm tab length given above is correct when the lower or supporting cover-glass is 25 mm square. When it is necessary to ring the mount, such as glycerine mounts, the top cover-glass should be round and 15 to 18 mm in diameter. For balsam or venice turpentine mounts, the square cover-glasses may be used on top. If a larger area is desired (fig. 20, C) a proper punch must be obtained for the correct size and shape of the opening in the metal slide. In that case, supporting cover-glasses of 25 mm width, by the desired length, may be used, together with suitable sized top cover-glasses (fig. 20, J, K). In that case the cardboard tabs must be shortened in order to fit the mount (fig. 20, I).

PUNCHING THE SLIDE

Suitable metal punches for making the opening in the metal slide are on the market. The one used by the writer is called the Challenge Eyelet Press No. 2 and makes a circular opening 13/16 of an inch in diameter. The punch should have a brass guide plate attached to its die table in order to keep the metal slide in proper position for punching. Figure 21, A shows the punched slide, together with details of the punch and brass guide plate mentioned above.

FORMING OR CRIMPING THE SLIDE

No suitable slide former is available through dealers, but a very satisfactory one has been constructed by Mr. Charles Bateman of the Division of Nematology, U. S. Bureau of Plant Industry. This is illustrated in figure 21. To operate the slide former, it is first necessary to close the short top lever, raise the long top lever and open the two side levers. The punched slide is then placed in proper position (fig. 21, B). The long top lever is then lowered, which makes the first crimp in the metal slide. While holding the long lever down, close the two side levers to make the second crimp (fig. 21, C). The two side levers are then released and the top lever raised. The short top lever is then released, the two sides of the long top lever pressed together, to release pressure on the finished slide and the slide is slipped off with the other hand (fig. 21, D). The metal slide is then ready for the insertion of the cover-glasses and cardboard tabs to make it complete (fig. 21, L).

Opuscula miscellanea nematologica, IV. G. Steiner, U. S. Bureau of Plant Industry.

(1) OBSERVATIONS ON NEMATODES IN BULBS OF AN IRIS TINGITANA HYBRID

A shipment of bulbous irises of the horticultural variety Wedgewood, imported from Beverwijk, Holland in October, 1935, was found by the inspector to be infested with nematodes and therefore submitted to the standard hot-water treatment (presoaked in water of 70-80° F. for 2½ hours and hot-water treated at 110½° F. for 1 hour). Later, fungus growth developed, the bulbs still harbored nematodes and were then submitted to the writer. Of the three species of nemas present one proved to be new and is here described as *Aphelenchoides limberi*, n. sp. It is named in recognition of the service which D. P. Limber of the Federal Inspection House, Washington, D. C., has rendered nematology in his work. He has brought to our attention much material of interest. Nemic associates of *Aphelenchoides limberi* in the present iris hybrids were *Aphelenchus avenae* Bastian, 1865, and a single specimen of a *Diplogaster* sp. In the bulbs so affected, *Aphelenchoides limberi* was the most numerous species. Petri dish cultures prepared with 2/3 cornmeal agar and 1/3 nutrient agar proved that the new species, as also *Aphelenchus avenae*, may feed on

the fungus, *Monilia sitophila* (Mont.) Sacc. growing on these plates. In the iris bulbs as well as in these cultures, males of *Aphelenchoides limberi* never were seen. The specimens of *Aphelenchus avenae* were remarkable because of their somewhat club-shaped tail (fig. 19, D) which at first seemed to indicate a new species. A scrutiny of the various specific characters, however, proved that the specimens were otherwise typical even to the number of striae (twelve) on the lateral fields.

Aphelenchoides limberi, n. sp. (fig. 22, A-C)

This new species has some resemblance to several of the species described by Gilbert Fuchs in 1930 and placed by him in the new genus *Parasitaphelenchus*, such as *P. cryphali* Fuchs, *P. pissodis piceae* Fuchs, *P. pissodis notati* Fuchs. The female of this new form, however, exhibits no characters which would exclude it from the genus *Aphelenchoides*, with which it is therefore grouped. It may be remarked that in our opinion the 3 aforementioned forms of Fuchs should also be removed to *Aphelenchoides*. Unfortunately Fuchs uses for 2 of them a trinomial designation, yet naming them species and not subspecies.

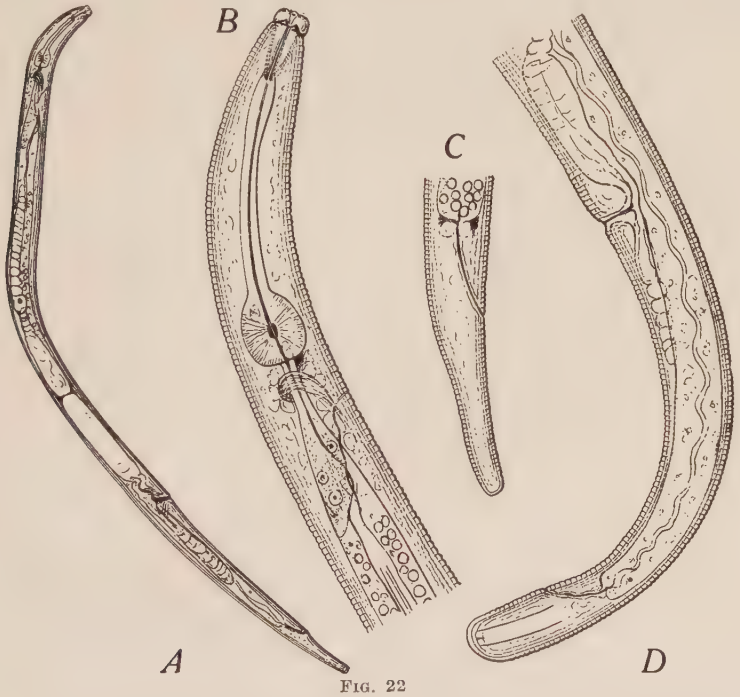

FIG. 22

A-C—*Aphelenchoides limberi*, n. sp. A—Female; X 160. B—Anterior end; X 690. C—Tail end of female; X 530. D—*Aphelenchus avenae* Bastian, 1865; posterior end of female with club-shaped tail; X 530.

Description.—Cuticle annulated; lateral fields about 1/7 as wide as body, with 4 fine longitudinal striae. Head well set off; buccal stylet distinct, not very strong, 11μ long, with distinct, but small basal knobs. Oesophagus typical, bulb with short, distinct valves. Intestine thin-walled, with double, alternating series of cells. Rectum about twice as long as anal body diameter. Tail somewhat finger-shaped, end obtuse to truncate. Prodelphic ovary outstretched, rather short. No postvulvar uterus. Vulvar lips quite distinct, vagina directed inward and forward.

Measurements.— ♀ : total length = .55 to .64 mm; a = 31.8(30.6 to 33.0), β = 7.8(6.5 to 9.2), γ = 15.3(14.5 to 16.0), v = 70%(66% to 72%).

Diagnosis.—*Aphelenchoides* dimensioned as stated, with syngonic (hermaphroditic or parthenogenetic?) reproduction. Male unknown. Female with coni-

cal, somewhat finger-shaped tail with obtuse or truncate end; lateral fields about 1/7 as wide as body, with 4 faint longitudinal striae. Buccal stylet long, with small, basal knobs.

Type locality.—The Netherlands.

Type host.—Bulbs of hybrid *Iris tingitana* Boiss. et Reut.

(2) ACROBELES VARIABILIS, N. SP. OBSERVED IN DISEASED IXIA BULBS

Ixia bulbs, heavily infested with an *Alternaria* sp. (fungus) were submitted from Jacksonville, Florida. These bulbs harbored numerous *Acrobeles* belonging to a new species. Their relationship to the disease is unknown.

Acrobeles variabilis, n. sp. (fig. 23, A-D)

Description.—From a superficial examination the species closely resembles *Cephalobus oxyuroides* de Man, 1876, at least in the size and shape of the body. However, head that of typical *Acrobeles*, having 2 circles of probolae, an inner of 3 low, bluntly rounded labial ones and an outer of 6 cephalic ones, each with a single seta; these cephalic probolae protruding less than labial ones, their setae arranged paired on dorsomedial and ventrosubmedial lines. Two submedial and one lateral cephalic papillae present, located outside of cephalic probolae. Of the 2 submedial papillae, one more anteriorly, the other more posteriorly placed (fig. 23). Amphid also seen, opening as small oral and transverse aperture. Pharynx and oesophagus as in the genus. Buccal plates rather weak except cheilorhabdions; dorsal metarhabdion with toothlet. Tail of female elongated, ending filiform, that of male shorter, conical and provided with a number of papillae, their arrangement as shown in figure 23. Phasmids present. Spicula curved, proximally cephalate. Gubernaculum not quite half as long as spicula. A pair of lateral membranes on otherwise strongly annulated cuticle.

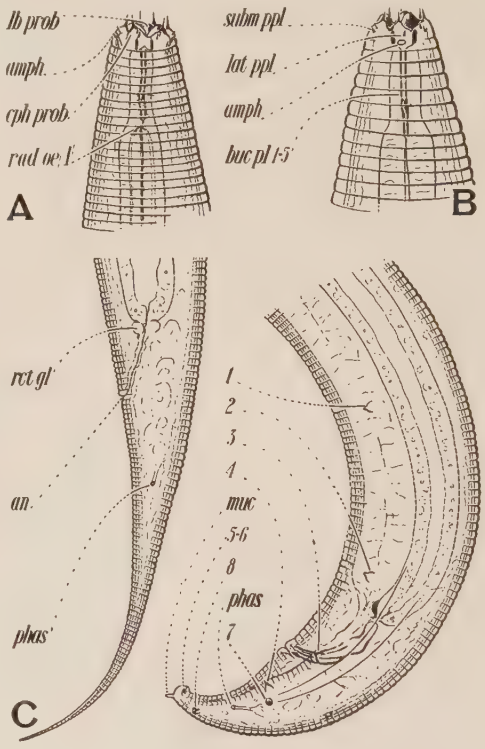

FIG. 23

Acrobeles variabilis, n. sp.

A—Ventral view of head end; *amph*, amphid; *cph prob*, cephalic probolae; *lb prob*, labial probolae; *rad oe l*, radii of oesophageal lumen; X 1,000. B—Side view of head end; *amph*, amphid; *buc pl* 1-5, buccal plates in 5 sets, beginning anteriorly with cheilorhabdions; *lat ppl*, lateral cephalic papilla; *subm ppl*, submedial cephalic papilla; X 1,000. C—Tail end of female; *an*, anus; *phas*, phasmid; *ret gl*, rectal glands; X 530. D—Tail end of male; *muc*, mucro; *phas*, phasmid; *1-8*, copulatory papillae; X 530.

Measurements.—♀ : total length = .65 to .67 mm; a = 17 to 17.7, β = 4, γ = 7.3 to 8, v = 60 to 61%. ♂ : total length = .65 to .67 mm; a = 18.6 to 18.8, β = 4 to 4.3, γ = 18.2 to 20.

Diagnosis.—*Acrobeles* with low, obtuse, labial probolae, with 6 asymmetrical cephalic probolae, grouped as follows: the 2 dorsosubmedial ones as a pair with setae toward dorsomedial, a lateral and a ventrosubmedial one as a pair with setae toward ventrosubmedial line. Tail of female elongated, ending filiform. Tail of male short, conical. Copulatory papillae of male as follows:

One ventrosubmedial about 3 times the length of the spicula in front of the anal opening, one similarly a short distance in front of the proximal end of the spicula, a third one just in front of the anal opening, a fourth at the first third of the tail, number 5 and 6 close to the tail end, 7th lateral in middle of tail and 8th dorsosubmedial a short distance in front of the tail end. Tail ending in short mucro.

Type locality.—United States (Florida).

Type association.—Ixia bulbs with fungus *Alternaria.*

This species was observed repeatedly, e. g. in bulbs of diseased *Lachenalia* sp. grown in a greenhouse, Arlington Farm, Va., in diseased bulbs of a paper-white polyanthus narcissus (*N. tazetta L.*) from Wilmington, N. C., and in a narcissus, var. King Alfred (*Narcissus pseudonarcissus L.*) from Castle Hayne, N. C.

(3) ACROBELES BODENHEIMERI, N. SP., A NEW NEMATODE FROM PALESTINE

Early in 1929 Dr. Bodenheimer of the Hebrew University, Jerusalem, Palestine, submitted to the writer 2 agar plates with nematodes apparently brought into the cultures on orange seeds from a seed bed. One of the cultures contained exclusively *Cephalobus elongatus* de Man, 1880, the other a new *Acrobeles* species, herein described. The former species is of a worldwide distribution.

Acrobeles bodenheimeri, n. sp. (fig. 21, A-F)

Description.—Tail of female conical, terminus broad obtuse; tail of male also conoid, but end thinner than in female and curved ventrad. Cuticle plainly annulated, with 2 lateral membranes. Head broad obtuse, not set off. Labial probolae low, broad obtuse; cephalic probolae 3, slightly concave, indicating subdivision into 2 parts, portions flanking dorsal and ventrosubmedial lines, ending in single setaceous point (fig. 24). Laterally 1, submedially 2, setose cephalic papillae. Amphids as shown in figure 24. Buccal armature with well developed cheilo-, pro-, meso-, meta- and telorhabdions but without any distinct toothlet. Oesophagus with short isthmus but well developed terminal bulb, containing full triple set of valves; transverse valves with 10 ribs. Terminus of ovary either outstretched caudad or reflexed a short distance; vulva prominent, but lips not protruded. Short sac-shaped posterior uterus present; viviparous (fig. 24).

Male with reflexed testis; copulatory papillae as follows: One about 3 times anal body width ventrosubmedial in front of anus, a second similarly halfway between this and anus, a third similarly level with anus, a fourth also ventrosubmedial near middle of tail, a fifth close to tail end, a sixth lateral near middle of tail and a seventh dorsosubmedial a short distance in front of tail end. Gubernaculum slightly curved, almost lineate, of about ½ spicula length. Latter curved, knife-shaped, proximal end broad, not cephalate. Six bursal muscles, most anterior one at beginning of ejaculatory duct. Phasmids in both sexes at about beginning of last third of tail.

Measurements.—♀ : total length = .8 to .9 mm; a = 15 to 16, β = 5. to 5.6, γ = 18 to 23, v = 67 to 70%. ♂ : total length = .69 to .75 mm; a = 14 to 16, β = 4.3 to 5., γ = 15 to 17.

Diagnosis.—Acrobeles, female and male with conoid, obtuse tail, with low, obtuse, undivided labial probolae, with 3 concave cephalic probolae, their sides ending in single setaceous point; viviparous; male copulatory papillae as figured.

Observations on life cycle.—Females copulate repeatedly. Eggs deposited uncleavaged; time from deposition to hatching 8 to 11 days; number of eggs produced at least 20; length of life 41 days or more.

(4) OBSERVATIONS ON TRICEPHALOBUS LONGICAUDATUS (BUETSCHLI, 1873)

The genus *Eucephalobus* was established in the preceding number of this journal for cephalobs with 3 lips (Opuscula Miscellanea Nematologica, III, p.

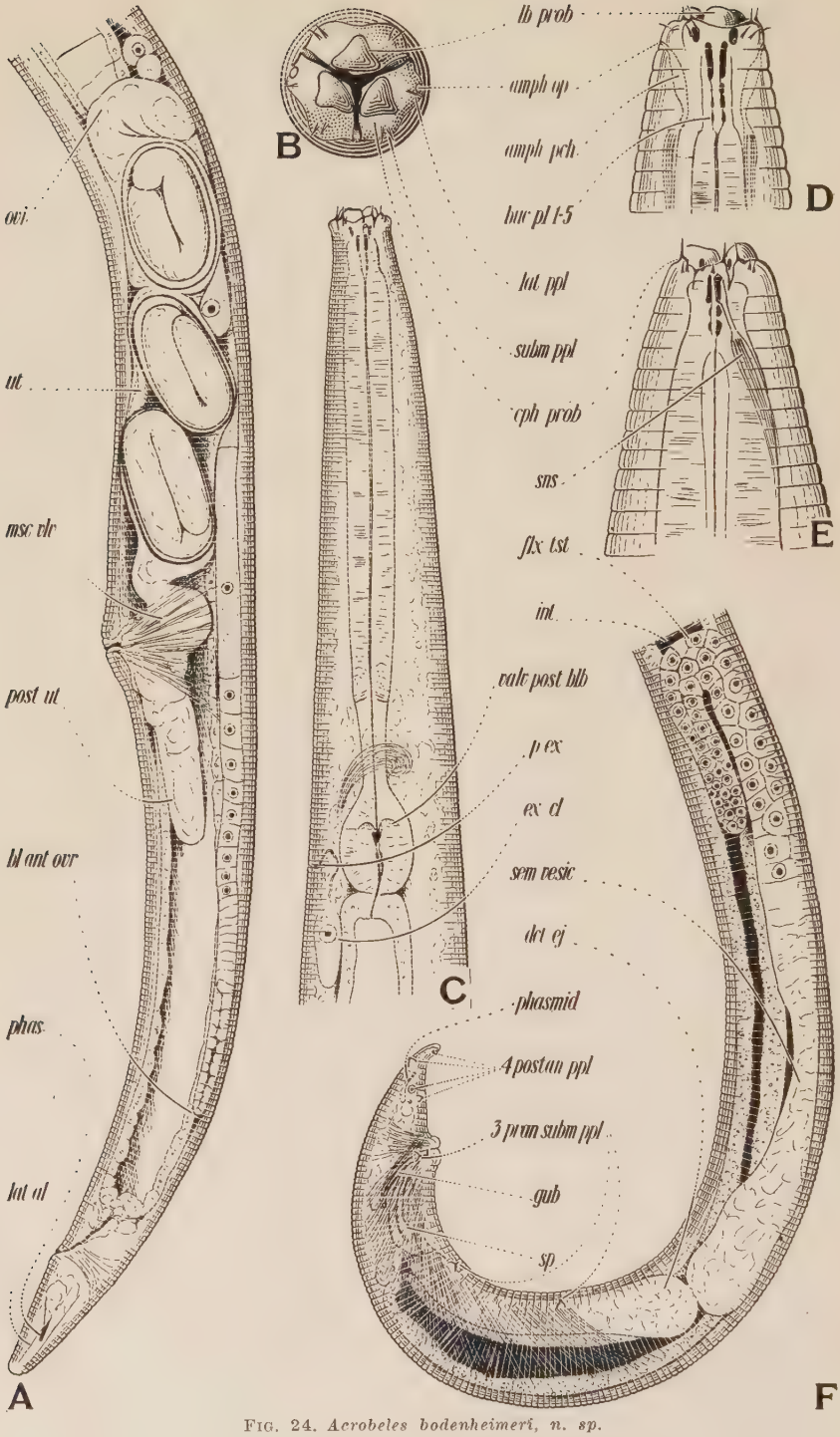

FIG. 24. *Acrobeles bodenheimeri, n. sp.*

21). By mistake *Eucephalobus oxyuroides* (de Man, 1876) was given as type species; however, *Cephalobus oxyuroides* de Man, 1876, has 6 lips and therefore can not be included with 3-lipped forms. Since the generic name *Eucephalobus* is now fixed on *Eucephalobus oxyuroides* (de Man, 1876), a new generic name must be provided for the cephalobs with 3 lips.

Tricephalobus, nov. gen.

Diagnosis.—Cephalobs with 3 lips.

Type species.—*Tricephalobus longicaudatus* (Bütschli, 1873). *Eucephalobus nannus* Steiner, 1936, now becomes *Tricephalobus nannus* (Steiner, 1936).

Tricephalobus longicaudatus (Bütschli, 1873)

Certain important characteristics of *T. longicaudatus* are not well known, particularly of the head and buccal cavity. The present observations are intended to fill this gap. *T. longicaudatus* is a species not infrequently seen, especially in or on dead, decaying insects. It feeds on bacteria and the decaying matter in which they occur. We observed the form recently in dead specimens of *Curculio auriger* Casey, the chestnut curculio, collected near Washington, D. C., by G. F. Gravatt of the Bureau of Plant Industry, and in beetles of *Listroderes obliquus* Gyll., the vegetable weevil, collected by M. M. High of the Bureau of Entomology and Plant Quarantine in Biloxi, Miss. The drawings were made after specimens from *Curculio auriger*. *E. longicaudatus* varies somewhat in the shape of male, as well as female tail and also in stoutness, older females being often very thick.

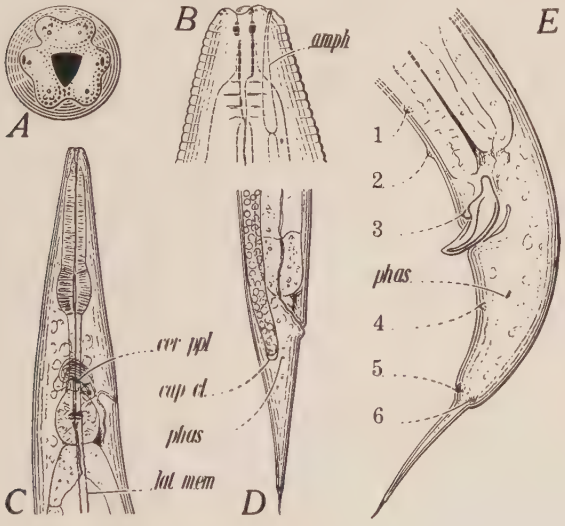

FIG. 25. *Tricephalobus longicaudatus* (Bütschli, 1873) A—Front view of head; X 1,400. B—Ventrosublateral view of head end; *amph*, amphid; X 1,400. C—Anterior end of body; *cer ppl*, cervical papilla; *lat mem*, lateral membrane; X 245. D—Tail end of female; *cap cell*, cap cell at end of ovary; *phas*, phasmid; X 245. E—Tail end of male; 1 ventromedial papilla; 5 ventrosubmedia papillae; 6 dorsosubmedial papillae; *phas*, phasmid; X 530.

Description. — Annulation of cuticle fine; lateral fields only about 1/15 to 1/16 as wide as body. Female tail long conical, usually ending in set off setaceous point; male tail arcuate, second half to last third most often sharply set off from rest by being more slender; terminus often as in female. Head

FIG. 24. *Acrobeles bodenheimeri*, n. sp.

A—Posterior end of female; *bl ant ovr*, blind end of anterior ovary; *lat al*, lateral membranes; *msc vlv*, vulvar muscles; *ovi*, oviduct; *phas*, phasmid; *post ut*, sac-shaped posterior uterus; *ut*, anterior uterus; X 350. B—Front view of head; *amph op*, opening of amphid; *cph prob*, cephalic probolae; *lat ppl*, lateral papillae; *lb prob*, labial probolae; *subm ppl*, submedial papillae; X 1,365. C—Anterior end of body; *ex cl*, cell of excretory apparatus; *p ex*, excretory pore; *valv post blb*, valvular apparatus of posterior oesophageal bulb; X 530. D—Dorsal view of head end; *amph op*, opening of amphid; *amph pch*, amphidial pouch; *buc pl*, 5 sets of buccal plates, cheilo-, pro-, meso-, meta- and telorhabdions; *lb prob*, labial probolae; X 1000. E—Side view of head end; *cph prob*, cephalic probolae; *sns*, sensilla of amphid; X 1,000. F—Posterior end of male; *dct ej*, ejaculatory duct; *flx tst*, flexure of testis; *gub*, gubernaculum; *int*, intestine; *phasmid*, phasmid; *4 post an ppl*, the 4 postanal papillae; *3 pran subm ppl*, the 3 preanal submedial papillae; *sem vesic*, seminal vesicle; *sp*, spicula; X 350.

not set off, front rather flattish, with 3 distinct lips, often somewhat concave, indicating a relationship to the six-lipped type. Cephalic papillae, as far as could be seen, 3 submedially and 2 laterally. Amphids as drawn in figure 25, B. Buccal armature as follows: Cheilorhabdions short, thick; prorhabdions most conspicuous; meso-, meta-, and telorhabdions weak, but location well marked by break in surrounding tissue. Corpus and middle oesophageal bulb strong, very muscular, terminal oesophageal bulb large, spherical, with full set of valves; transverse valves with 6 ribs. Intestine consisting of 4 series of cells. Rectum about as long as anal body width. Cervical papillae as well as phasmids seen. Vulva not protruding; vagina very short; uterus single, small, slightly passing vagina posteriorly; no trace of a posterior uterus; oviduct long, separated from uterus by an almost spherical portion interpreted as glandular and furnishing the eggshell. Ovary reflexed, end reaching usually past anus into lumen of tail; whole female apparatus to right of intestine; no special receptaculum seminis. Male sexual apparatus to right of intestine, with reflexed testis; spicula arcuate, short, wide, proximal end not capitate; gubernaculum half as long, also arcuate, narrow, distal portion lineate. Copulatory papillae as follows: Single ventrosubmedial one about level with or close to proximal end of spicula; 4 submedial ones, first about 1½ times spicula length in front of anus, second slightly anterior to anus, third about middle, fourth at end of thick portion of tail; single dorsosubmedial papilla at end of thick portion of tail.

Measurements.—♀: total length = .450 to .630 mm; a = 12.7 to 21, β = 3.8 to 6.3; γ = 8.5 to 11; v = 55 to 63%. ♂: total length = .45 to .524 mm; a = 17 to 21.8; β = 4.2 to 5.1; γ = 9.5 to 10.8; spicula = 23μ.

(5) REMARKS CONCERNING ACROBELES CROSSOTUS STEINER, 1929

The dimensions of this species are here reported, having been omitted in a previous paper (1929, Ztschr. Morph. u. Okol. Tiere, 15: 547-558). ♀: total length = .6 mm; a = 17; β = 3.7; γ = 14.3; v = 60%. ♂: total length = .66 mm; a = 21; β = 3.4; γ = 14.3

These specimens were collected from diseased bulbs of iris var. Imperator submitted by a dealer in Philadelphia, Pa., probably grown in California.

On the classification of the Tylenchinae. I. N. Filipjev, Branch of the Academy of Sciences of USSR, Almata, Kazakstan, USSR.

Among the species of the genus *Anguillulina* Gervais et van Beneden, 1859 (= *Tylenchus* Bastian, 1865), there are included species of very diverse structure, namely:

(a) The head may contain a differentiated cuticular framework or may be devoid of such.

(b) The spear may be very large, of medium size, or feebly developed, completely disappearing in the adults of some closely related genera.

(c) The esophagus may be tylenchoid, i. e., with esophageal glands inside its walls and well differentiated from the intestine, or aphelenchoid with the esophageal glands outside its walls, the boundary of esophagus and intestine then being indistinct.

(d) The ovaries may be double or single.

(e) The bursa may be adanal, not reaching the tip of the tail, or caudal, embracing its end.

(f) The cuticle may be striated more or less coarsely.

These characters, together with others, are taken as a basis for a new generic subdivision here proposed. All genera bearing a bursa without true papillar ribs are mentioned.

A. Genera with head chitinized, cuticle coarsely striated, spear strong but not very long, tail varying in size, bursa always caudal.

Rotylenchus Filipjev, 1934. Esophagus aphelenchoid, ovaries double, Type: *Rotylenchus robustus* (de Man, 1880), new comb. (= *Tylenchus robustus* de Man, 1880). Other species: *R. obtusus* (Bastian, 1865), *R. multicinctus* (Cobb, 1893), *R. africanus* (Micoletzky, 1916),

R. bradys (Steiner et LeHew, 1933), all new combs. All with short tail, mostly rounded in female. Related species with long tail: *R. similis* (Cobb, 1893), *R. apapillatus* (Imamura, 1931), both new combs. and possibly synonymous.

Hoplolaimus Daday, 1905. Characters as in *Rotylenchus* but without lateral caudal papillae in male. Type: *Hoplolaimus tylenchiformis* Daday, 1905. Other species: *H. coronatus* Cobb, 1923.

Pratylenchus Filipjev, 1934. Esophagus aphelenchoid, ovary single. Type: *Pratylenchus pratensis* (de Man, 1880), new comb. (= *Tylenchus pratensis* de Man, 1880). Other species: *P. mahogani* (Cobb, 1920), *P. musicola* (Cobb, 1919), *P. sacchari* (Soltwedl, 1881), *P. dendrophilus* (Marcinowski, 1909), *P. aberrans* (Thorne, 1935), all new combs.

Tylenchorhynchus Cobb, 1913 (syn. *Bitylenchus* Filipjev, 1934). Esophagus tylenchoid, ovaries double. Type: *Tylenchorhynchus dubius* (Bütschli, 1873), new comb. (= *Tylenchorhynchus cylindricus* Cobb, 1913). Other species: *T. macrurus* (Goodey, 1932), *T. gracilis* (de Man, 1880), *T. zostericola* (Allgen, 1934), *T. magnicauda* (Thorne, 1935), all new combs. Questionably included: *Tylenchus styriacus* Micoletzky, 1922, *Tylenchus symmetricus* Cobb, 1914, *Tylenchus alatus* Cobb, 1930, *Tylenchus lamelliferus* de Man, 1880.

Chitinotylenchus (Micoletzky, 1921) Filipjev, 1934. Esophagus tylenchoid, ovary single, spear bifurcated behind. Type: *Chitinotylenchus paragracilis* (Micoletzky, 1921), new comb. (= *Tylenchus* (*Chitinotylenchus*) *paragracilis* (Micoletzky, 1921). Another species: *C. annulata* (Cassidy, 1930), new comb.

B. Head without chitinization, cuticle finely striated (with some exceptions), esophagus tylenchoid.

a. Ovaries double.

Dolichodorus Cobb, 1914. Spear very long, bursa lobate, head set off. Type: *Dolichodorus heterocephalus* Cobb, 1914.

Tetylenchus, gen. nov. Spear of moderate size. Males unknown. Type: *Tetylenchus tenuis* (Micoletzky, 1921), new comb. (= *Tylenchus tenuis* Micoletzky, 1921). Other species: *T. clavicaudatus* (Micoletzky, 1921), *T. granulosus* (Cobb, 1893), all new combs.

b. Ovary single.

1. Bursa adanal, tail long in both sexes, spear well developed. *Tylenchus* Bastian, 1865. Spear with basal swellings. Type: *Tylenchus davainii* Bastian, 1865. Other species: *T. agricola* de Man, 1881, *T. filiformis* Bütschli, 1873, *T. bryophilus* Steiner, 1914, *T. minutus* Cobb, 1893, *T. leptosoma* de Man, 1880, *T. terricola* Bastian, 1865, *T. farwicki* Rahm, 1924, *T. weidenbachii* Rahm, 1924, *T. graciloides* Micoletzky, 1925, *T. uniformis* Cobb, 1893, *T. arboricolus* Cobb, 1922, *T. emarginatus* Cobb, 1893, *T. turbo* Marcinowski, 1909, *T. velatus* Bütschli, 1873, *T. oryzae* de Haan, 1902. A related species with coarse transverse and longitudinal striation of the cuticle: *T. costatus* de Man, 1921 (= *T. cancellatus* Cobb, 1925). Doubtfully included: *T. foliicola* Zimmerman.

Halenchus Cobb, 1933. Characters as in *Tylenchus* but with nearly plain degenerate esophagus and tail with a hook on the end, marine. Type: *Halenchus fucicola* (de Man, 1892) Cobb, 1933 (= *Tylenchus fucicola* de Man, 1892). Another species: *H. mediterraneus* (Micoletzky, 1922) Cobb, 1933.

Psilenchus de Man, 1921. Characters as in *Tylenchus* but with a plain spear. Type: *Psilenchus hilarulus* de Man, 1921.

Eutylenchus Cobb, 1913. Should probably be placed here, female unknown. Male coarsely annulated, with 4 cephalic setate and a striated adanal trapezoidal bursa. Type: *Eutylenchus setiferus* Cobb, 1913.

2. Bursa caudal, spear short with basal swellings.

Ditylenchus Filipjev, 1934. Body elongate, spicules narrow, cephalated. Free-living or plant-parasitic species, not degenerate. Type: *Ditylen-*

chus dipsaci (Kühn, 1857), new comb. (= *Tylenchus dipsaci* (Kühn, 1858) Bastian, 1865). Other species: *D. angustus* (Butler, 1913), *D. intermedius* (de Man, 1880), *D. procerus* (Bally et Reydon, 1931), *D. radicicola* (Greeff, 1872), *D. sycobius* (Cotte, 1920), *D. major* (Fuchs, 1914), *D. gallica* (Steiner, 1935), *D. pinophila* (Thorne, 1915), *D. phyllobius* (Thorne, 1934), *D. graminophila* (Goodey, 1933), all new combs. Doubtfully included: *Tylenchus brevicauda* Micoletzky, 1925, *Tylenchus durus* Cobb, 1922, *Tylenchus eurycephalus* de Man, 1921.

Anguina Scopoli, 1777 (= *Anguillulina* Gervais et van Beneden, 1859). Female with short large body. Male with short flat spicules. Degenerate plant-parasitic species. Type: *Anguina tritici* (Steinbuch, 1799), new comb. (= *Vibrio tritici* Steinbuch, 1799). Other species: *A. agrostis* (Steinbuch, 1799), *A. graminis* (Hardy, 1850), *A. millefolii* (Lőw, 1874), *A. cecidoplastes* (Goodey, 1934), all new combs. Doubtfully included: *Tylenchus balsamophilus* Thorne, 1926.

Iotonchium Cobb, 1920. Body short, spear extremely minute, degenerate species parasitic in fungi. Type: *Iotonchium imperfectum* (Bütschli, 1876) Cobb, 1920.

C. Genera with degenerate spear (the absence of the spear must be regarded as secondary, in the larvae probably it is present).

Neotylenchus Steiner, 1931. Body long, esophagus tylenchoid with a faint bulb. Males unknown. Plant parasites. Type: *Neotylenchus abulbosus* Steiner, 1931. Other species: *N. consobrinus* (de Man, 1906), *N. fungorum* (Bütschli, 1873), both new combs.

Hexatylus Goodey, 1926. Characters as in *Neotylenchus* but without a trace of a bulb, esophagus aphelenchoid (?). Males unknown. Plant parasites. Type: *Hexatylus viviparus* Goodey, 1926. Goodey assumes the identity of *Neotylenchus* and *Hexatylus*. He may be right if *H. viviparus* is more advanced in degeneration and goes through a Neotylenchus-like stage during development. These two genera may be close to the prototype of *Ditylenchus*.

Hemicycliophora de Man, 1921. Extremely long and slender, male with a wing-like adanal bursa. Female unknown. Type: *Hemicycliophora typica* de Man, 1921. This genus may be an offshoot of *Tylenchus*.

Macroposthonia de Man, 1880. Body short, coarsely annulated, esophagus aphelenchoid, spicules large, true bursa apparently absent. Type: *Macroposthonia annulata* de Man, 1880. Probably a male of *Paratylenchus bukowinensis* Micoletzky, 1921, in which case the generic name *Macroposthonia* has priority.

D. Spear with basal swelling, body extremely long and slender, with a wing-like adanal bursa, cuticle said to be smooth.

Ecphyadophora de Man, 1921. Type: *Ecphyadophora tenuissima* de Man, 1921. The bursa indicates a relationship to *Tylenchus* and *Dolichodorus*, the smooth cuticle casts some doubt on the inclusion of the genus in the Tylenchinae.

MINUTES

One hundred seventy-fifth to one hundred eightieth meetings

The 175th meeting was held December 21, 1935. Dr. Emmett W. Price was elected resident vice-president to represent the Society in the Washington Academy of Sciences. Dr. G. F. Otto was relected to the editorial committee of the Proceedings. Papers or notes were presented by Dikmans, Hall, Ewing and Lucker.

The 176th meeting was held January 18, 1936. Papers or notes were presented by Steiner, Jones and Hall.

The 177th meeting was held February 15. Papers or notes were presented by Chitwood, Dikmans, Cram and Thorne (the last mentioned paper read by Steiner).

The 178th meeting was held March 21. Papers or notes were presented by Chitwood, Dikmans and Ewing.

The 179th meeting was held April 18. Report of the committee on the Ransom Memorial Fund was read by Dr. Dikmans. Miss Jocelyn Tyler was elected to membership in the Society. Papers or notes were presented by Justin Andrews, Cort, Sandground, Steiner and Kerr.

The 180th meeting was held May 16. E. B. Cram, G. Dikmans, G. F. Otto, E. W. Price and G. Steiner were elected to constitute a Board of Trustees for the Ransom Memorial Fund. Professor H. J. Bennett, Dr. A. O. Foster and Dr. D. A. Porter were elected to membership in the Society. Papers or notes were presented by Chitwood, Bartsch, Schwartz and Horsfall (the last mentioned paper read by Price).

WENDELL H. KRULL, Recording Secretary.

THE BRAYTON H. RANSOM MEMORIAL TRUST FUND

Whereas Dr. Brayton H. Ransom has rendered valuable services to humanity by his contributions to the study and control of parasites; and

Whereas the Helminthological Society of Washington deemed it fitting that the memory of Dr. Ransom should be perpetuated, and in furtherance of this purpose appointed a committee composed of Ch. Wardell Stiles, Maurice C. Hall, Eloise B. Cram and W. W. Cort to solicit funds to be used to establish a suitable memorial; and

Whereas friends and colleagues of Dr. Ransom, residing in all parts of the world, have contributed as original donors a total sum of $1,020.93 with the understanding that the contributions should be used to create a memorial in honor of Dr. Ransom, said original donors being as noted on the attached list.

And whereas, at the time of soliciting funds no definite form of memorial had been decided upon, but suggestions were solicited from the original donors and contributions were received with the understanding that the nature of the memorial to be established would be dependent on the total sum collected, keeping in mind the wishes of a majority of the donors; and whereas there has been no substantial agreement as to the form that this memorial in honor of Dr. Ransom should take except that it should be of a kind to encourage the study and advance of the Science of Parasitology and related sciences;

NOW, THEREFORE, in order to establish such a memorial, the present committee in charge of said funds nominated in October, 1935, consisting of G. Steiner, chairman, Paul Bartsch, Eloise B. Cram, G. Dikmans and G. F. Otto, as members, joined by M. C. Hall, C. W. Stiles and W. W. Cort, the surviving members of the original committee with the exception of Eloise B. Cram who is on the present committee, at the direction of the Helminthological Society, hereby gives, transfers, assigns and sets over, and delivers to Eloise B. Cram, G. Dikmans, G. F. Otto, E. W. Price and G. Steiner, TRUSTEES, named by the Helminthological Society to act as such, the sum of one thousand two hundred ninety-nine dollars and 91 cents ($1,299.91), (contributions paid $1,020.93, interest accrued $290.73, expenses to date $11.75), representing all the funds collected plus interest thereon up to the present date, minus expenses to date, IN TRUST, HOWEVER, for the purposes and uses described below; and the above named Trustees in consideration of the foregoing, hereby accept these funds subject to these said trusts and uses, and agree to carry out the terms of this Trust.

1. This trust fund is to be known as the BRAYTON H. RANSOM MEMORIAL TRUST FUND.

2. There shall be five Trustees, but any three of the Trustees agreeing on any subject matter shall have power and authority to transact any Trust business at any time and their action shall be binding on the Trust Fund and on all the Trustees. Trustees who are absent from a meeting shall be notified promptly of any action taken during their absence. In the event that some action by the Trustees is neeessary and it is impossible to have a meeting, the Trustees may individually and separately and without consultation with other Trustees indicate their will in writing properly signed and witnessed and the action of three or more Trustees shall be binding on the Trust Fund and all Trustees.

3. Three or more of the Trustees shall have authority and power to draw checks and to indorse checks in the name of the Trust Fund, without including the names of the other Trustees, nor shall it be necessary at any time for three or more of the Trustees to join the names of the other Trustees when executing any paper or instrument on behalf of the Trust Fund.

4. A majority of the Trustees shall name one of the Trustees to act as Secretary and Treasurer. The Trustee so designated shall keep a set of books showing investments, income, disbursements as well as all other information relating to the finances of the Trust. The secretary shall also keep a record

of the meetings, the matters handled at such meetings and their disposition. He shall also record any action taken upon other affairs relating to the Trust Fund which may have been brought up and acted upon without having a formal meeting. These books and records shall be open to inspection by the other Trustees at any time during reasonable hours. In the event that the Trustees deem it more expedient to have separate individuals to act as Secretary and Treasurer, they may name one of their number to each post.

5. The Trustees, as soon as practical, shall take the funds herein transferred to them and invest the same in what appears to the Trustees to be a safe manner. No restriction is placed on the type of investment the Trustees may make, and they shall not be personally liable for mistakes of judgment. Any funds in their hands insufficient in quantity to be readily invested may be placed on deposit in a bank or other safe depository, in the name of this Trust, and shall be subject to withdrawal on the signature of three or more Trustees in the name of the Trust. If it is deemed expedient, a checking account may also be opened.

6. The Trustees of this fund shall use the income thereof (or so much of said income as they deem advisable) to encourage and promote the study of and advance of the Science of Parasitology and related sciences. But in NO case may they use the principal.

The principal of this Trust Fund shall be deemed to consist of: FIRST, the original contributions hereby transferred to the Trustees, but not the income such contributions have earned while in the hands of the committee, except such portion as may be specifically designated by the Trustees; SECOND, all subsequent contributions; THIRD, all past and future earnings of existing funds which shall have been set aside to be added to the principal by formal act of the Trustees.

The particular use to be made of the income shall be discretionary with the Trustees, as long as that use fulfills the general purpose of perpetuating the memory of Dr. Ransom and the advancement of the Science of Parasitology and related sciences. This use may take the form of presenting a prize or prizes at intervals, either in the form of money or a suitable medal for a notable contribution to this field of science, the publishing of worthy writings relative to this science, the subsidizing in part or in whole of a journal or other publication devoted in toto or in part to this field, the dissemination of knowledge, granting of scholarships or any other means which appear appropriate to the Trustees to accomplish the general and international purposes of this Trust. (The intent of the foregoing enumeration is to suggest possibilities and is not to be treated as a limitation.)

7. Because of the fact that the fund is small at present, it is suggested that not all the income be used for these purposes, but that a part of the income be kept and re-invested so that the principal may be increased, in order that the ultimate usefulness and importance of the fund may be increased. The Trustees are specifically authorized to accept further contributions.

8. The Trustees, upon creation of this Trust, shall notify all original donors of such action by publication in the Proceedings of the Helminthological Society of Washington, a copy of which is to be sent to all donors that can be reached.

9. The Trustees may meet whenever necessary but are required to meet at least once every year, and the use of the income for the past year shall then be discussed and determined and a report of their action published in the Proceedings of the Helminthological Society of Washington, or some other appropriate journal in the event the former ceases to be published. It is suggested that the Trustees set a fixed date for such annual meetings.

10. In the case of the death or resignation of a Trustee, a successor Trustee shall be elected by a majority of the remaining Trustees, it being intended that this body of Trustees shall be self-perpetuating.

11. The foregoing instructions are in the nature of instructions for the

guidance of the Trustees and it is the intent of this instrument that in no case shall any party dealing in good faith with said Trustees in relation to the Trust Fund be obliged to see to the application of the funds or be obliged to see that the terms of this Trust are complied with or be obliged to inquire into the necessity or expediency of any act of said Trustees or be obliged to inquire into the terms of this trust agreement.

IN WITNESS WHEREOF WE HAVE HEREUNTO SET OUR HANDS AND SEALS AT WASHINGTON IN THE DISTRICT OF COLUMBIA, THIS 17TH DAY OF JUNE, 1936.

Witness the signature of W. W. Cort this 13th day of June, 1936.

(Seal) Leonard O. Engle,
Notary Public.

District of Columbia,
June 17, 1936

Subscribed and sworn to before me this 17th day of June, 1936, by all members of the previous and present Committee and the Trustees of the Ransom Memorial Trust Fund except W. W. Cort.

(Seal)

W. E. Taylor,
Notary Public in and for
the District of Columbia.

Commission expires July 14, 1936.

COMMITTEE

Ch. Wardell Stiles
W. W. Cort
Maurice C. Hall
Eloise B. Cram
Paul Bartsch
G. Dikmans
G. F. Otto
G. Steiner

TRUSTEES

Eloise B. Cram
G. Dikmans
G. F. Otto
E. W. Price
G. Steiner

DONORS

Ackert, J. E.
Andrews, Justin M.
Animal Husbandry Division, B. A. I., U. S. D. A.
Anonymous
Ashford, Bailey K. (deceased)
Avery, L.
Babcock, O. G.
Bancroft, Nebraska, Women's Club
Bartsch, Paul
Bass, O. C.
Baylis, H. A., London, England
Becker, E. R.
Benbrook, E. A.
Bengston, J. S.
Bigelow, R. P.
Biological Survey, U. S. D. A.
Bishopp, F. C.
Bozicevich, John
Brumpt, E., Paris, France
Buhrer, Edna M.
Butler, C. S.
Cahn, A. R.
Chandler, A. C.
Christie, J. R.
Ciurea, J., Bucharest, Roumania
Clark, Herbert C.
Cobb, Grace Sherman
Cobb, N. A. (deceased)
Colton, H. S.
Copelan, S. L.
Cort, W. W.
Cotton, C. E.
Coventry, F. A.
Cram, Eloise B.
Curtice, Cooper
Danheim, Bertha
Davis, H. S.
Day, L. Enos
Dikmans, G.
Dove, W. E.
Dreyer, E. C.
Ewing, H. E.
Faust, E. C.
Fibiger, Johannes (deceased), Copenhagen, Denmark

Fuelleborn, F. (deceased), Hamburg, Germany
Galli-Valerio, B., Lausanne, Switzerland
Gedoelst, L. (deceased), Brussels, Belgium
Goldberger, Joseph (deceased)
Graybill, H. W.
Hadley, F. B.
Hadwen, S., Toronto, Canada
Hall, Maurice C.
Hartman, E.
Hartnack, Hugo
Hegner, R. W.
Hickman, Jessie
Higgins, M. E.
Hoeppli, R., Peiping, China
Hoffman, W. A.
Hogue, M. J.
Holliday, C. H.
Houck, U. G. (deceased)
Jacocks, W. P., Delhi, India
Jägerskiöld, L. A., Göteburg, Sweden
Jones, M. F.
Joyeux, Ch., Marseille, France
Juday, Chauncey
Kincaid, T.
Laboratory of Parasitology, Facultatea de Medicina, Veterinara, Bucharest, Roumania
Lambert, S. M., Suva, Fiji
LaRue, G. R.
Latham, Vida A.
Linton, E.
Lynch, K. M.
Lyon, M. W.
McIntosh, A.
MacNeal, W. J.
Magath, T. B.
Mahood, S. A.
Marsh, C. T. N.
May, H G. (deceased)
Metcalf, M.
Middleton, Austin R.
Miller, F. W.
Miller, Harry M.
Ministry of Agriculture of Roumania
Neave, S. L.
Nelson, Dr.
Neveu-Lemaire, M., Paris, France
Nichols, H. J. (deceased)
Nighbert, E. M.
Nolan, M. O.
North American Veterinarian
Otto, G. F.
Pearse, A. S.
Raffensperger, H. B.
Railliet, A. (deceased), Alfort, France
Ransom, Anna M.
Ransom, G. H.
Riley, W. A.
Rivas, D.
Rosen, Hilda
St. John, J. H.
Sandground, J. H.
Sawyer, W. A.
Schroeder, E. C. (deceased)
Scott, John W.
Shapiro, L. (deceased)
Shattuck, G. C.
Shaw, Louise C.
Shigley, J. F.
Shillinger, J. E.
Shipley, Arthur E. (deceased), Cambridge, England
Smith, Clara W.
Smith, Theobald (deceased)
Steele, Frank John
Steiner, G.
Stoll, N. R.
Stunkard, H. W.
Sweet, Winfield C.
Theiler, Sir Arnold, Pretoria, Union of So. Africa
Thorne, Gerald
Underhill, B. M. (deceased)
Ward, H. B.
Wenrich, D. H.
White, G. F.
Whitmore, E. R.
Wigdor, M.
Yokogawa, S., Taihoku, Taiwan, Japan

PLEDGES

Allen, E. A.
Andrews, J. S.
Harwood, P. D.
Krull, W. H.
Lynch, V. E.
Price, E. W.
Roe, G. C.
Schwartz, B.
Sellers, F. F.
Shorb, D. A.
Strong, R. P.
Wright, W. H.
Yoshida, S.
Young, W. H.

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